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NOTES ON SOME DICRANOPHORINAE (ROTIFERA)[1]

G. M. NEAL

Abstract

The reliability of certain structures of rotifers used for specific identification is discussed. *Dicranophorus uncinatus* (Milne) of Harring and Myers has been redesignated as *D. aquilus* (Gosse) and both species are redescribed. *D. uncinatus* of Weber (nec Milne) is a member of the *D. rostratus-grypus* group and *D. cernuus* Harring and Myers is a synonym of *D. rostratus* (D.-N. & F.), while *D. corystis* H. & M. and *D. haueri* H. & M. are both valid species distinct from *D. rostratus.*

The taxonomy and nomenclatorial status of several of the species of the genus *Dicranophorus* are in a state of confusion owing largely to the fact that either the illustrations of earlier authors were inadequate, their descriptions did not agree with their illustrations, or the descriptions were vague. This has been recognized by Harring and Myers (8, p. 679) when they refer to a number of named species of the genus *Dicranophorus*, "Some of these are perfectly good species, but the majority may perhaps as well be considered useless baggage; this applies to the species described by Ehrenberg, Schmarda, Hudson and Gosse, Bergendal, and Glascott." Similar views are voiced by de Beauchamp (1, p. 328), "Mieux veut rejeter formallement toutes ces vielles espéces, ainsi qu'un certain nombre de Gosse, de Miss Glascott et d'autres." A consideration of some of these earlier names leads to the conclusion that they must be regarded as invalid since they cannot be applied with certainty to any known species. This results in confusion and disagreement by authors as to the identity of a given species. These points are clearly illustrated in the consideration of *Dicranophorus aquilus* (Gosse) and *D. uncinatus* (Milne) presented in this paper.

In considering these earlier figures and descriptions it is well to be clear as to what characters are of taxonomic significance in the group considered. Among characters which have been used in the genus *Dicranophorus* to distinguish species have been the jaws, the toes, the presence or absence of eyes, the presence or absence of a rostrum, presence or absence of various sense organs, and size of the animal.

Many members of this genus are, superficially, very similar in the shape of the body and in the length and shape of the toes. If we consider two

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Contribution fom the Department of Zoology, University of Toronto, Toronto, Ontario.

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specimens, the toes may be of equal length and of the same general shape, a rostrum may be present as well as eye spots; unless one sees the jaws one cannot be certain whether the two specimens are of the same species or not. If one examines the jaws and these are different in the two specimens considered, then one may conclude that one has two different species; if one does not see the jaws, one remains in doubt as to the specific status of the two specimens.

In many instances eyes are difficult to observe, particularly if the eyes lack pigment, or if they contain red pigment this is frequently bleached out in the preserved specimens. The toes, jaws, and rostrum, then, remain the major key characters in specific identification.

Although rotifers appear to be remarkably constant morphologically, actually little is known for certain as to the extent of intraspecific variation, either genetic or environmental; thus whether every variation noted, in any one species, should have varietal rank is open to question. If a variation develops within a population of rotifers we would expect that amictic reproduction would tend to preserve this variation, where such a variation were not to the detriment of that population. In very few species, for example, some *Keratella* and *Brachionus*, do we have any conception of the range of specific variation related to seasonal variations. It becomes evident, therefore, that controlled experiments are necessary to determine the extent and nature of variations in the Rotifera as in other groups of animals.

Below will be considered some of the characters of members of the genus *Dicranophorus* that are used for specific identification:

1. *The Jaws*

Of the characters which have been used to distinguish species the jaws appear to be the most diagnostic. Some of the apparent variations noted are probably to be accounted for on the basis of the amount of pressure applied in mounting, or a difference in the orientation of the jaws. This may be the explanation of the difference between Pawlowski's (13) and Donner's (3) figures of *Dicranophorus nikor*; the former omits the supramanubria while the latter shows them.

In correspondence, Wulfert states in reference to his description of the intramallei of *D. longidactylum* as being round, "I don't believe now, that the last point is of any importance." The size and relation of the intramallei to the other structures of the jaw appear to have some taxonomic importance. The shape, as indicated, may be varied to some extent, by pressure or orientation of the jaws while one is studying them.

Wulfert (20, p. 407, Fig. 2*a–d*) gives an illustration of a variation of *Dicranophorus robustus* which he names var. *europaeus* in which, in addition to the toes being straight instead of S-shaped, the jaws are asymmetrical; in one specimen, however, from another European locality the jaws are symmetrical. The same author has found variation in the jaws of *Encentrum saundersiae* (Hudson), some being longer and more compressed than in the typical form.

In many species of the Rotifera the jaws may serve as distinctive specific characters. This is particularly true of those possessing the forcipate type of jaws. However, minor variations in structure for certain species have been noted—some of these are a result of flattening of the jaws by mechanical pressure on the cover slip or a different orientation of the jaws while they are being studied. In comparing the structure of jaws it would seem desirable that one have the same orientation as shown by other workers. In some instances some of the variations noted are actually structural. If these definite structural variations are constant throughout a population or occur in sufficient numbers they may merit subspecific or infrasubspecific rank; but as stated above, before any stand can be taken on their taxonomic rank more study is needed on the range of variation of taxonomic characters between and within a species.

2. *The Toes*

The ratio between toe length and body length as well as the shape of the toes remains surprisingly constant. These characters are usually sufficiently distinct to be of some taxonomic importance. Questions sometimes arise as to the significance of minor variations in the length and shape of the toes within a clone or between different local populations. In *Cephalodella gibba* (Ehr.) for example, I have found specimens with straight toes, one-third to one-half the length of the usually long and recurved toes. Both types are found in the same pond; specimens with the longer recurved toes are more common at the beginning of the season and those with the shorter, straighter ones later, although an occasional long-toed individual is also found later in the season. Present knowledge does not warrant a decision as to whether they represent distinct species or merely seasonal forms of the same species. Wulfert (20) has found that the European form of *Dicranophorus robustus*, to which he has given the name *europaeus*, differs from the American form in having straight instead of S-shaped toes. Most of his specimens, in addition, had asymmetrical jaws (see above). It would seem apparent, then, that the ratio of toe length to body length, as well as the shape of the toes, may be subject to some variation within a species.

3. *The Eyes*

What is the taxonomic significance of the occasional absence of eyes in forms which do not differ in other respects from common and generally recognized species?

It is known that eyes are present in the free-swimming larval stage of many sessile rotifers but are lost after the larvae become attached. Such larvae have been reported as distinct species before the complete life history of such forms was known.

It is possible that the absence of eyes in rotifers is, in itself, of no more taxonomic significance than is blindness; for example in mice. In some cases the reported absence of eyes may be due to difficulties of observation. Eyes are difficult to see where they naturally lack pigment or where the red

pigment has been bleached out in preservation. My own observations confirm the absence of eyes in *Dicranophorus uncinatus* (Milne) as reported by Milne (12) and Wulfert (20). Zawadowsky (21) figures an individual which in all other respects appears to agree with *D. uncinatus* except in the possession of very small red eyes. In the latter instance, I believe these represent variations or abnormalities rather than deviations of taxonomic significance.

Some of the disagreement on the presence or absence of eyes in a particular species appears due to inaccurate identification of species, for example *Diglena uncinata* Milne as figured by Weber (18) (see below).

4. *Sense Organs*

The presence or absence of various sense organs has been used for taxonomic purposes but their occurrence at times appear to be unreliable; for example, the presence of setae at the end of the foot has been reported in *D. uncinatus* by Milne and shown in his drawing (Plate 2, Fig. 2); he states that they are difficult to see. Donner (3, Fig. 25) shows them in *D. nikor*. It may be that these setae not only are difficult to see but also are easily broken off, which may account for the fact that Wulfert did not note the setae in *D. longidactylum*. Again, it is possible that they might be present in some specimens and not in others. Wulfert, in correspondence, says that he has noted another sense organ (overlooked by other workers) on the appendix (tail) of *D. longidactylum* and suggests the possibility of there being two sense organs, one at the base of the toes and the other on the "appendix".

5. *Size*

There is a characteristic range of size within each species of rotifer which may differ from locality to locality and also, to some extent, within the same collection.

Some of the differences in size reported are due no doubt to the fact that some authors (Wulfert) have measured living specimens while others have measured specimens that were first narcotized and then placed in preserving fluid, or measured specimens placed directly into preserving fluid. Size in the last instance, owing to greater contraction, will be smaller than in either of the other two, and in the second case, smaller than the first owing to the slight amount of contraction that takes place when the narcotized animal is placed in preserving fluid.

From the above consideration of the characters used in determining the specific status of members of the genus *Dicranophorus* one can see that some allowance must be made for the range of variations that occurs in these characters within any one species. Before creating a new species or form less than specific rank, then, the new character must have a significant range of variation from the mean to warrant this action. To obtain an estimate of the significance of the range of variation of this new character a sufficient number of specimens must be treated by some statistical means as suggested

by Mayr *et al.* (11, p. 153, *et seq.*) keeping in mind that with most species of rotifers so few individuals are obtained "that only the simplest statistics can be used; e.g., size, including range if several specimens are available, and simple proportions and ratios."

Status of *Dicranophorus aquilus* (Gosse) and *Dicranophorus uncinatus* (Milne)

Gosse's name (*Diglena aquila*) has appeared in several publications. Different authors have taken different features of Gosse's description as a basis for the identification of their specimens. It may be concluded that, at least in some cases, the specimens while agreeing in some respects with Gosse's description differed in others. If, therefore, all of the characters possessed by all of the specimens to which Gosse's name has been applied were combined, the resulting animal would differ markedly from the specimen described by Gosse as *D. aquila*. It is probable that if Gosse had given a good figure of the jaws and toes of the animal to which he gave the name *aquila* (such as Milne did for *D. uncinatus*) much of the present confusion would have been avoided. Unfortunately earlier authors did not appreciate the importance of the jaws as taxonomic characters.

The following is Gosse's description of *D. aquila*. The present author's comments have been placed in parentheses.

"Body fusiform (not so in his figure). Head with beak (a number of other species have a beak or rostrum); foot short, thick (in common with most *Dicranophorus*); toes nearly as long as trunk, thick to ½ the length then diminished to stiff straight rods with obtuse points (Gosse's figure shows the toes less than ½ the length of the body. The narrowing to obtuse points is not clearly indicated.) No eyes are present." Later, however, Gosse mentions observing "a specimen with a very large black occipital eye, if indeed, it were not an opaque chalk mass of the brain." (Eyes are frontal in *Dicranophorus.*) At times one finds in *D. uncinatus* the duct of the subcerebral glands filled with dark granules. Could the specimen found by Gosse with the "dark opaque chalk mass" be *D. uncinatus*, and confused by him with the one he described as *D. aquila?*

If one examines Harring and Myers (8, Plate 36) one finds figures of *D. uncinatus* (Milne), *D.* sp. H. & M. and on Plate 31, *D. aspondus* H. & M. In all of these species there is a rostrum, the toes are over one-half the length of the body and as straight as shown in Gosse's figures. The jaws differ in all of them.

De Beauchamp (1) gives a figure of jaws of what he calls *Diglena aquila*. This figure resembles the figure of the jaws given by Harring and Myers (8) for *D. uncinatus*. It would appear, then, that de Beauchamp and Harring and Myers had the same species under observation, if we consider the jaws alone. De Beauchamp describes his animal as having eyes at the region where the ducts open from the retrocerebral sac; Harring and Myers, on the

other hand, state that their specimen was eyeless, as was Gosse's and as was also Milne's. The jaws shown by Harring and Myers are not those of *D. uncinatus* (Milne) as figured by Milne.

What can be seen of the jaws in Gosse's figure shows the manubria bowed inwards as in *D. uncinatus* according to Milne's figure, whereas in the figures of Harring and Myers and of de Beauchamp the manubria are turned outwards at their tips.

The similarity of the jaws illustrated by Harring and Myers and by de Beauchamp has been noted by Hauer (9) and by Fadeew (5). The latter states that *D. uncinatus* H. & M. is equal to *D. aquilus* (Gosse) and this conclusion is probably based on de Beauchamp's figure of the jaws of the specimen he describes as *D. aquilus* (Gosse). Again, though the two specimens of Harring and Myers and of de Beauchamp seem to agree in other respects, there is still the question of the eyes. Lack of life history studies of any member of the genus *Dicranophorus* prevents us stating definitely whether eyes are absent at any time during the seasonal cycle or in any members of a clone.

From the above discussion it can be seen then that, of the specimens in question, *D. uncinatus* (Milne) is a recognizable and valid species and *D. uncinatus* as figured by Harring and Myers is another species confused by them with Milne's species; that the jaws of de Beauchamp's specimen and that of Harring and Myers agree in structure but the two forms differ in the presence of eyes in the one and the absence of eyes in the other; that Gosse's figure is inadequate and what can be determined of the jaws is not in agreement with the figures given by Harring and Myers and by de Beauchamp; that, further, the toes of Gosse's specimen do not agree with those figured by Harring and Myers nor does Gosse's figure agree with his description of the toes.

The question now is whether the example figured by Harring and Myers as *D. uncinatus* (Milne) should be given a new name or considered to be *D. aquilus* (Gosse) and whether de Beauchamp's animal also should be considered as *D. aquilus*. We may concede that Gosse's animal is inadequately described and that Harring and Myers' form needs a new name. The following alternatives seem to present themselves; either we may use Harring and Myers' description and figure as a completed description and their figures as good figures of Gosse's *D. aquilus* or we could place *aquilus* in the invalid list of species and give a new name to *D. uncinatus* as figured by Harring and Myers. I propose to follow the first course; this would not be an unusual procedure but would follow along the lines used by Harring and Myers in designating a description to a species named by an earlier author where the description is inadequate and the figures are useless, for example *D. forcipatus* (Muller) as indicated by Harring and Myers (8) on pages 696 and 697.

On this basis I give below the description of *D. uncinatus* (Milne) and *D. aquilus* (Gosse) with figures of the latter from Harring and Myers (7).

DICRANOPHORUS UNCINATUS (Milne) (Fig. 6) (whole animal from slightly contracted specimen), Fig. 2 *a, b, c* (after Wulfert (20)).

Dicranophorus longidactylum Fadeew. (Cited by Wulfert, 1936).
Dicranophorus longidactylum Fadeew. Wulfert, 1936, p. 408, fig. 4–4*b*.
Dicranophorus uncinatus (Milne). Hauer, 1936, p. 138, Pl. 2, Fig. 15.
Diglena uncinata Milne. Milne, 1885. Pl. II, Figs. 1, 2, 8.
Diglena uncinata Milne. Hudson & Gosse, 1889, Suppl. Pl. XXXIII, Fig. 13–13*b*.
non *Dicranophorus uncinatus* (Milne). Harring and Myers, 1928, p. 745, Pl. 36, Figs. 1–2.
non *Diglena uncinata* Milne. Weber, 1898, p. 496, Pl. 19, Figs. 15–18.
non *Diglena uncinata* Milne. Zawadowsky, 1926, Fig. 22 (after Weber, 1897).
non *Arthroglena uncinata* (Milne). Voigt, 1912, p. 111, Fig. 209 (after Weber, 1897).
non *Arthroglena uncinata* (Milne). Montet, 1915, p. 329.
Pleurotrocha uncinata (Milne). Zawadowsky, 1926, Fig. 21.
Dicranophorus uncinatus (Milne). Fadeew, 1929.
(?) *Dicranophorus nikor* Pawlowski. Pawlowski, 1938, p. 126, Fig. 1, Table IX, Fig. 2.
(?) *Dicranophorus nikor* Pawlowski. Donner, 1951, p. 637, Fig. 25.
Diglena aquila Gosse. Weber and Montet, 1918, p. 132.

Milne (12) described and figured as *Diglena uncinata* a rotifer which is the same animal figured by Wulfert (20) as *Dicranophorus longidactylum* Fadeew. This is not the same animal as described and figured by Harring and Myers (8) as *Dicranophorus uncinatus* (Milne). In Fadeew's paper (4) quoted by Wulfert (20) there is no mention of *Dicranophorus longidactylum*. Wulfert may have had another reference to Fadeew, if so, I have not been able to locate it. It is possible he may have given the wrong authority. In Fadeew's check list (5) *D. uncinatus* is mentioned but not *D. longidactylum*. If Fadeew had described *D. longidactylum* I feel certain it would have been mentioned, as the other species described by him in his 1927 paper are included in the check list of 1929. Myers (personal interview) admitted that the animal described by Harring and Myers (8) is not Milne's animal but is another species. Harring and Myers' species is a true species in need of a name.

Description of D. uncinatus (Milne)

The integument is quite firm and tends to hold its shape.

Dorsally, the body appears somewhat cylindrical; in a lateral view it is distinctly gibbous dorsally. The foot is short and broad; the tail clearly set off from the body. The toes are long, and about one-third the total length of the entire animal; they have a wide base, are slightly S-shaped, and end in sharp points.

A constriction separates the head from the rest of the body. The corona is oblique, though not as ventral in position as in Harring and Myers' figures.

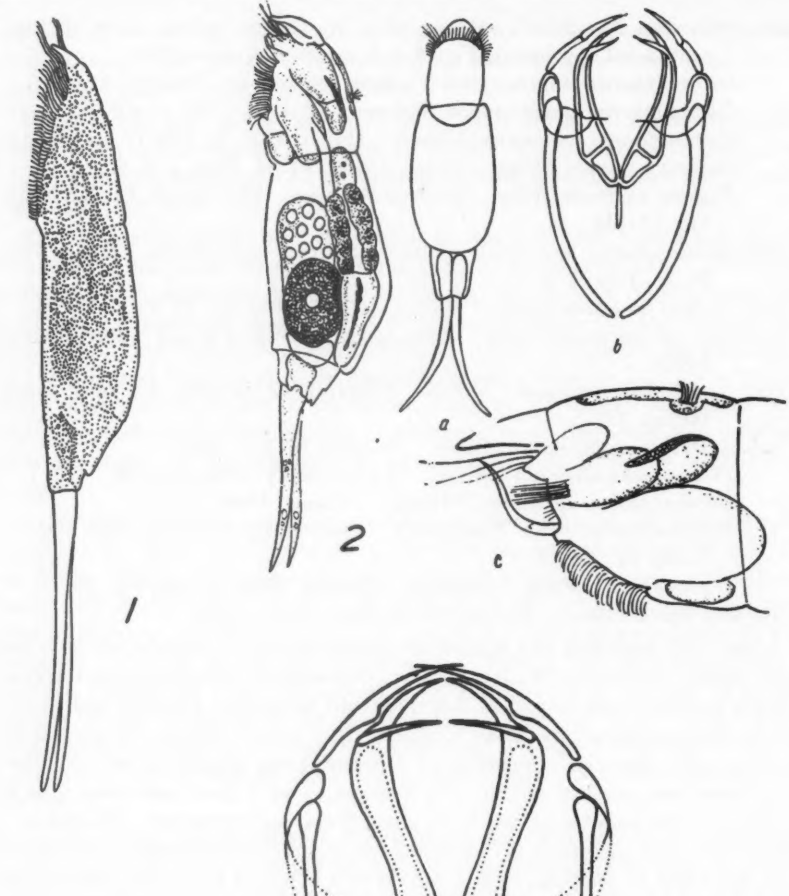

FIG. 1. *Dicranophorus aquilus* (Gosse) (after Harring and Myers (7) Plate 36, Fig. 1). Lateral view.

FIG 2. *Dicranophorus uncinatus* (Milne) (after Wulfert (20) Fig. 4–4*c*). Whole animal, lateral view; *a*, dorsal outline; *b*, trophi; *c*, head with rostrum, cirri, and antenna.

FIG. 3. *Dicranophorus aquilus* (Gosse) (after Harring and Myers (7) Plate 36, Fig. 2). Trophi.

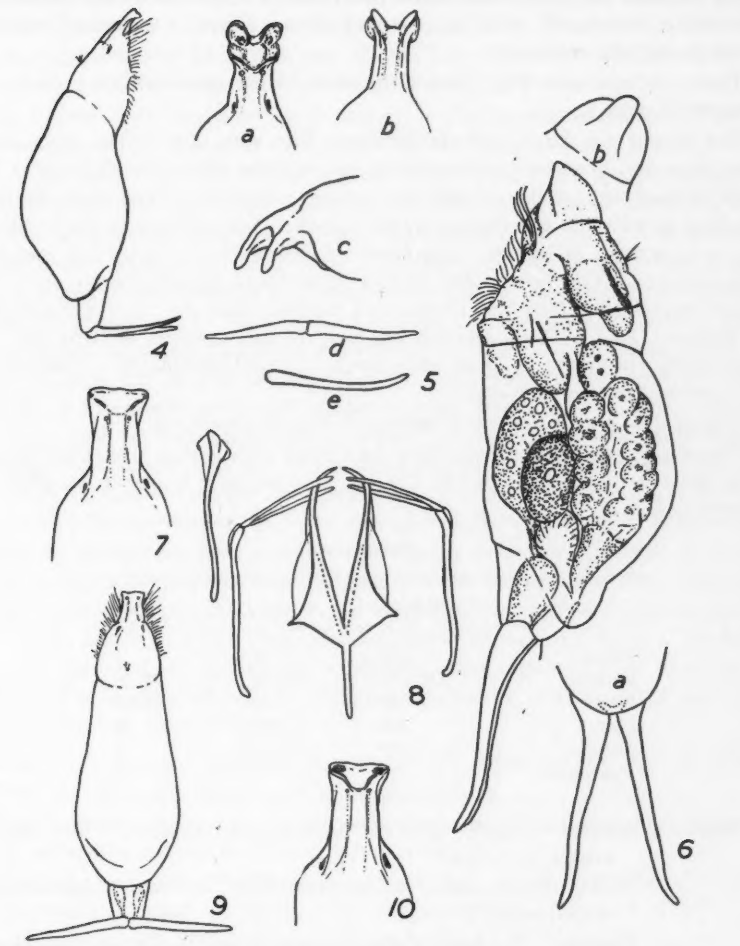

FIG. 4. *Dicranophorus rostratus* (Dixon-Nuttall and Freeman). Specimen No. 1, lateral outline.

FIG. 5. *Dicranophorus rostratus*. (Third example.) *a*, dorsal view of rostrum; *b*, ventral view of rostrum; *c*, lateral view of rostrum; *d*, toes; *e*, toes of second specimen, similar to *D. cernuus* H. & M.

FIG. 6. *Dicranophorus uncinatus* (Milne). Lateral view (drawn from a slightly contracted specimen). *a*, dorsal view of toes; *b*, dorsal view of the hood.

FIG. 7. *Dicranophorus rostratus* (Dixon-Nuttall and Freeman). Rostrum of first specimen, showing slightly chiselled front edge.

FIG. 8. *Dicranophorus rostratus*. Ventral view of the trophi and lateral aspect of a manubrium.

FIG. 9. *Dicranophorus rostratus*. Dorsal view showing slight indication of lateral projections at the tip of the rostrum.

FIG. 10. *Dicranophorus rostratus*. Dorsal view of the rostrum and dense patches to indicate thickenings where lateral pegs are contracted on the ventral side.

At its anterior end the corona bears two bundles of cirri-like cilia, the rest of the corona is covered with long, strong cilia. There is a distinct rostrum which is slightly recurved.

There are no eyes (?). (See note above with reference to presence or absence of eyes.)

The trophi are large and distinctive. The rami are widest just above their base and posteriorly are slightly incised just above the fulcrum. The ends of the rami are drawn out into a long, evenly-narrowed main tooth at the base of which is a strong secondary tooth. The fulcrum is short without any enlargement at its tip. The unci are thin and are drawn out into long acute points. The intramallei (Milne calls these muscle loops) are broad plates rounded laterally and attenuated medially into points (supramanubria of Wulfert (20)). The manubria are long, bowed inwards at their tips, and broadened anteriorly at their attachment to the intramallei. There is no enlargement on the free end.

The gastric glands are round spherical-shaped structures.

There is a retrocerebral sac with a duct filled with dark granules (at times?). This feature of the granules in the sac and its duct is shown in Wulfert's figures (2*a*, *b*, *c*).

DIMENSIONS

Author	Total length	Toes	Jaws
Milne	254 μ	84 μ	? μ
Wulfert	230–280	50–84	28–30
Hauer	100	67	19–20
Neal	200	50	30
Donner	190	43	22
Pawlowski	213	40	20

Habitat: Wulfert—weakly alkaline "Graben und Quellen"; late fall and winter; not rare.
Neal—pH 7.8, found in Ceratophyllum, in May, Kingston region of Ontario.
Donner—"Buchenlaubstreu" near a brook in the zoo at Schleswig.
Milne—from the neighborhood of Glasgow.

Discussion

Milne's and Wulfert's figures agree fairly well. Milne's figure is a little more dumpy and the gibbous nature of the dorsal side more pronounced, as though the drawing were made from a slightly contracted specimen. The jaws are very similar, the intramallei slightly larger but of the same general shape. Hauer's figure shows only the jaws. These are similar to those of Milne. As Hauer has pointed out (9, p. 138), "The jaws agree in certain ways with those figured by de Beauchamp (1) and H. & M. (8) (see comments above). The intramalleus has a different shape than is shown by the above

authors, the plate is shorter and drawn out into a short but distinct point medially." Hauer's description and figure apply to *D. uncinatus* (Milne). As he suggested and as Myers (see above) has confirmed, the figures of de Beauchamp and Harring and Myers are those of another animal.

Pawlowski (13) described and named a *Dicranophorus nikor*. This is very similar to *D. uncinatus* (Milne). In the figure of the jaws (p. 126, Fig. 1) the manubria and the fulcrum are longer than in *D. uncinatus* figured by Hauer, Wulfert, or Milne. Further, the toes in the whole animal (Plate IX, Fig. 2) are shown shorter and decurved and not slightly S-shaped as in *D. uncinatus*. Donner (3, p. 637, Fig. 25) gives a figure of *D. nikor* Pawlowski. This has toes similar in shape to *D. uncinatus* (Milne), though they are slightly shorter in proportion to the length of the body. The jaws are similar with the exception that the manubria are similar to those in Milne's figure of *D. uncinatus* and not as long as indicated by Pawlowski for his animal. The fulcrum is intermediate in size between those indicated by Milne for *D. uncinatus* and by Pawlowski for *D. nikor*. Donner shows the intramallei and their extensions into the supermanubria; the latter are not shown by Pawlowski. The difference in shape between the supramanubria in the drawings of Donner and of Wulfert could quite easily be the result of pressure. The slight differences in the jaws do not seem to warrant specific rank for this form (*D. nikor*) as has been indicated in the discussion above. Donner states that two long bristles are found at the base of the toes, as mentioned previously, Milne mentions these also and shows them in his figure. Wulfert did not observe them, but did note another sense organ on the "appendix" (tail). As mentioned above, the presence or absence of these setae does not seem to warrant specific status.

Donner's form seems halfway between *D. uncinatus* (Milne) and *D. nikor* Pawlowski. The differences can be stated thus:

Toes: Donner's animal shows the same shape as *D. uncinatus* but shorter; Pawlowski's similar to Donner's but straighter.

Manubria: longer in Pawlowski's animal than in *D. uncinatus* (Milne), Donner's *D. nikor*, or Wulfert's *D. longidactylum*.

Fulcrum: longer in Pawlowski's animal.

Intramallei: not clearly shown by Pawlowski, nor are their extensions into the supramanubria. These structures are shown by Donner in his figure for *D. nikor*. The difference in shape between these structures in Wulfert's and Donner's figures could be the result of pressure.

With regard to the length of the toes, this has been considered above and the differences do not seem to make for the validity of the species *D. nikor* as a distinct species from *D. uncinatus*.

The variations indicated above would seem to me to fall within the limits of natural variation and would not warrant the formation of a new species of *D. nikor*.

DICRANOPHORUS AQUILUS (Gosse) Figs. 1 and 3 (after Harring and Myers (8))

Dicranophorus uncinatus (Milne), H. & M. 1928, pp. 745–747, Pl. 36, Figs. 1–2.

Diglena aquila Gosse. Gosse, P. H., 1887, p. 865, Pl. 14, Fig. 10.

Diglena aquila Gosse. H. & G., 1889; Pl. XXXIII, Figs. 20*a*–*b*, Suppl.

Dicranophorus aquila (Gosse). Fadeew, 1929.

Diglena aquila Gosse. De Beauchamp, 1914; p. 328, Fig. 4.

Dicranophorus uncinatus (Milne). Remane, 1932; p. 212, Fig. 192 No. 4.

This species was described by Harring and Myers (8) as *Dicranophorus uncinatus* (Milne).

Comparing *D. uncinatus* and *D. aquilus*: *D. aquilus* has a slenderer body, less gibbous dorsally; the head is longer, constriction absent, and the corona more ventrally placed, almost in the body axis; the foot and tail are more pronounced than in *D. uncinatus*. The toes are longer, slenderer, and lack the slight S-shaped curve of *D. uncinatus*. The trophi of *D. aquilus* differ in a number of respects from those of *D. uncinatus*; the rami are longer and at half their length noticeably narrowed; the secondary tooth of each ramus is longer and more needlelike; the unci have a knoblike enlargement beyond half their length which is absent in *D. uncinatus*; the intramallei are smaller, more narrowly triangular and the supramanubria are not broad plates as in *D. uncinatus*; the manubria are longer and curved outward toward their tips where they end in a slight enlargement; in *D. uncinatus* they curve inward without the terminal enlargement.

The ducts of the retrocerebral sac are rudimentary and do not contain the granules seen in *D. uncinatus*. (Gosse mentions one of his specimens as having a black chalky mass at the back of the brain (see above).)

Eyes are absent (?) (see de Beauchamp (1) and above discussion.)

DIMENSIONS

Author	Total length	Toes	Jaws
Harring and Myers	225 μ	90 μ	30 μ
Hudson and Gosse	$\frac{1}{85}$ in.	One-half length of body	

Habitat: Harring and Myers report this species from acid water (pH 4.0–6.4) near Egg Harbour, New Jersey, and Lake Wood, Mt. Desert Island.

I have found it in a collection made from Borthwick Lake, an acid bog lake, in northern Ontario.

De Beauchamp found it in the "gours of Garonne, near Toulouse".

Gosse's material came from Ireland.

My specimens, found in moss and *Utricularia* samples taken on June 18, 1938, were badly contracted so that measurements could not be made.

Notes on Weber's (18) Figure of *Diglena uncinata* Milne

Diglena uncinata Milne. Weber and Montet, 1918, p. 130.
Diglena uncinata Milne. Weber, 1898; p. 496, Pl. 19, Figs. 15–18.
Diglena uncinata Milne. Zawadowsky, 1926; Fig. 22 (after Weber).
Arthroglena uncinata (Milne). Voigt, 1912; p. 111, Fig. 209 (after Weber).

Weber (18) figures a *Dicranophorus* under the name *Diglena uncinata* Milne. His description is taken from Hudson and Gosse (10, Suppl.) but his figure is original and the description and the figure do not agree. This figure is reproduced by Voigt (15) under the name *Arthroglena uncinata* (Milne), and by Zawadowsky (21) as *Diglena uncinata* Milne. Harring and Myers (8) include these as synonyms for *Dicranophorus uncinatus* (Milne). There are certain characters of Weber's animal that do not agree with Milne's animal, but places it in with the *Dicranophorus rostratus* group. These characters are as follows:

1. Weber shows two eye-spots as being present; Milne did not observe eyes in his animal, *D. uncinatus*, nor did Wulfert, Pawlowski, nor Donner. Zawadowsky (21) shows a figure of another animal (Fig. 21) named *Pleurotrocha uncinata* (Milne) which agrees in form of body and toes, etc., with Milne's figure of *D. uncinatus*. He states that eyes are present but are much smaller than those found in the animal *Diglena uncinata* after Weber (18). Zawadowsky gives Weber's figure for comparison with his own figure of *Pleurotrocha uncinata* and considers them synonymous.

2. The toes of Weber's specimen differ from those of Milne's. The toes of Zawadowsky's *Pleurotrocha uncinata* agree with Milne's figure as also do those of Wulfert's (21) animal. The toes of Weber's figure are closer to those of Harring and Myers (8) for *D. uncinatus* (*D. aquilus* see above) but there is something about the set of them in the figure and the general shape of the body that is remotely reminiscent of *D. grypus* H. & M. (8, p. 37, Figs. 7–9). In Weber's figure the toes are shown with a peculiar shouldering (claws) near the tips of the toes, which is not evident in any other figure of *D. uncinatus*.

3. The jaws are not very well illustrated by Weber but there appear to be *two* uncial teeth such as is shown by Harring and Myers (8, Plate 37, Figs. 2–3) for *Dicranophorus cernuus* or *D. grypus* (Plate 37, Figs. 7–9) the latter having longer toes than *D. cernuus*. The rami are slightly broader than in *D. uncinatus*.

4. The one feature in Weber's animal agreeing with Milne's figure is the presence of the long cilia on the corona. In Dixon-Nuttall's figure of *D. rostratus* long hairs are also shown toward the dorsal limits of the corona but not as little tufts as in *D. uncinatus*.

5. The presence of two uncial teeth, shown in his figure, but not agreeing with his description, is the most significant character in deciding that *D. uncinatus* as figured by Weber is some species, other than *D. uncinatus* (Milne) and belonging to the *Dicranophorus rostratus-grypus* group.

Status of *Dicranophorus rostratus* (Dixon-Nuttall and Freeman) and Related Forms

Diglena rostrata Dixon-Nuttall and Freeman. Dixon-Nuttall and Freeman, 1901–03; pp. 215–216, Pl. 9, Figs. 1–3.

Dicranophorus cernuus Harring and Myers. H. & M., 1928; Pl. 37, Figs. 1–3.

Dicranophorus cernuus H. & M. (8, Plate 37, Figs. 1–3) is *D. rostratus* (D.-N. & F.). The slight differences do not seem to me to warrant specific rank for Harring and Myers' animal. One difference given by Harring and Myers is that there are no "rostral pegs" present in *D. rostratus*—in Dixon-Nuttall's figure distinct lateral extensions are shown on either side of the tip of the rostrum, which I take to be the rostral pegs that are mentioned by Harring and Myers. The difference in appearance of the pegs may be one of drawing and seem too slight for specific rank. The other difference stated by Harring and Myers is that the jaws of *D. rostratus* have "peculiarly elongate, triangular rami". The lateral lamella of the rami, shown in Harring and Myers are indicated in Dixon-Nuttall and Freeman and need to be widened slightly to give the same appearance as in Harring and Myers' figure. All these differences would fall within the limits of natural variations discussed above. It would seem to me, therefore, that *D. cernuus* H. & M. is a synonym for *D. rostratus* (D.-N. & F.).

DIMENSIONS

Author	Total length	Toes	Jaws	State of animal
Neal	220 μ	54 μ	20 μ	Slightly contracted
Neal	197	45	22	Two-thirds contracted
Neal	216	60	33	Slightly contracted; eye small
Dixon-Nuttall and Freeman	240	53	—	
Harring and Myers	200	45	30	

Habitat: Neal—from Borthwick Lake, an acid water bog lake in the Patricia District of northern Ontario, Canada. From moss and *Utricularia.*

Dixon-Nutall and Freeman—from the locality of Knowsley Park, Lancashire, England.

Harring and Myers—from acid water pH 6.8 and less, from Atlantic City, Mt. Desert Island in Maine, and Oneida and Villas Counties in Wisconsin, U.S.A.

I found some variations in specimens of *D. rostratus* collected from Borthwick Lake. The collections were made from moss and *Utricularia.* The samples were narcotized and then fixed with osmic acid. There was some contraction which to some extent may account for the smaller size of the animals, compared with the results of other authors, but according to Myers,

who saw some of the collections from Borthwick lake, the size of all the rotifers in the collections were somewhat smaller than in any of the collections he had seen from other localities.

The toes were shorter than given by Myers but in general proportions nearer those of Dixon-Nuttall and Freeman. The toes varied slightly in shape from almost straight to approximately the shape shown by Harring and Myers (8) for *D. cernuus* (Fig. 4, 5*e*, *g*). No joint could be seen on any specimens examined. At times the toe was twisted on itself—this was probably a reaction to the preservative.

The jaws (Fig. 8) were approximately the same as given by Harring and Myers (8). It is easy to understand how Dixon-Nuttall and Freeman overlooked the thin lateral lamellae of the rami—they are very thin and showed up best at a magnification of 1000 × with careful adjusting of the light.

The rostrum shows some variation in size and shape. In Fig. 7 the rostrum is similar to that shown by Dixon-Nuttall and Freeman. Viewed dorsally, one notes on either side at the anterior tip of the rostrum a thickened circular opaque region (Fig. 10). In lateral aspect these are seen to be ventrally directed small lateral extensions of the rostrum. Preserving may have caused some contraction in the ventral rather than lateral direction of the extensions. In another specimen, Fig. 5*a–d*, the anterior end of the rostrum appeared laterally fluted and again from the lateral view one could discern the projections—in this specimen these were about twice as long as in the first. In a third specimen the toes were similar to *D. cernuus* H. & M. (Fig. 5*e*), the jaws were slightly larger than in the other specimens and the eyes smaller than in the other two specimens examined.

Note on *Dicranophorus corystis* H. & M.

Dicranophorus corystis H. & M. Harring and Myers, 1922; p. 555.
Dicranophorus corystis H. & M. Harring and Myers, 1928; p. 727, Pl. 33, Figs. 1–3.
Arthroglena rostrata D.-N. & F. Von Hofsten, 1909; p. 21, Fig. 3.
(?) *Dicranophorus rostratus* var. *corystis* (H. & M.). Fadeew, 1927; Table 1, Fig. 12.
(?) *Dicranophorus rostratus* var. *corystis* (H. & M.). Fadeew, 1929.

Hofsten (16) figured a rotifer which he named *Arthroglena rostrata* (D.-N. & F.). He shows jointed toes but does not think this is sufficient for specific separation. "Joint on toes was overlooked by the English authors, or it was weakly developed in the specimens observed or it was absent entirely" (16). He compares his animal with *D. uncinatus* (as figured by Weber, see above) and *D. lutkeni* but in both cases his comparison ends with the toes and hood; no comparison is made of the jaws, which differ markedly in all the above species. The jaws of *D. corystis* H. & M. are quite different from those of *D. rostratus* (D.-N. & F.); in the former there is one uncial tooth, in the latter two uncial teeth.

Fadeew (4) in his figure of *D. rostratus* var. *corystis* (H. & M.) shows the rostrum but no figure of the jaws. As Fadeew's animal had the jointed toes and lateral projections to the rostrum, it does not necessarily follow that Fadeew had the same species as is shown in Hofsten's paper, since Fadeew does not make any comparison of the jaws.

Hofsten (17) "refutes" Harring and Myers' (7) statement that his specimen is a distinct species and insists his is identical with *D. rostratus* (D.-N. & F.). This scarcely can be supported when we consider the differences—*toes jointed*, relatively longer, and pointed; the lateral projections on the rostrum relatively longer; but above all the jaws are distinctly different. The detail of the rami, such as lateral lamellae, may not be shown in Hofsten's figure, but there is but a single uncial tooth shown.

It is seen, then, that *Dicranophorus corystis* H. & M. is not identical with *D. rostratus* (D.-N. & F.) and remains as a true species till relationship may be shown to one of the other jointed-toed, lateral-lobed-rostral forms.

DIMENSIONS

Author	Total length	Toes	Jaws
Von Hofsten and Fadeew	Up to 300 μ		
Harring and Myers	225	60 μ	35 μ

Habitat: Von Hofsten and Fadeew—from sphagnum bogs.
Harring and Myers—common in acid water.

Note on *Dicranophorus haueri* H. & M.

Dicranophorus haueri H. & M. 1928; p. 725, Pl. 30, Figs. 4–5.

Dicranophorus rostratus var. *corystis* (H. & M.). Fadeew, 1927; Table 1, Fig. 12.

Dicranophorus rostratus var. *corystis* (H. & M.). Fadeew, 1929.

Fadeew (5) gives this as a synonym for *Dicranophorus rostratus* var. *corystis* (H. & M.) (see above). The jaws of *Dicranophorus haueri* are quite different from those of *Dicranophorus rostratus.* The rami have double interlocking teeth at their tips, and the unci are single-toothed with a knoblike enlargement for articulation with the rami. The eyespots are small in contrast to the obvious ones found in *Dicranophorus rostratus.*

Harring and Myers (8, p. 725) mention the fact that they had received a sketch of this species from Fadeew which might indicate that the animal he calls *D. rostratus* var. *corystis* could be *D. haueri* if one had a figure of the jaws. Fadeew, apparently, did not make any comparison of the jaws in his animal; whether he sent drawings of the jaws to Harring and Myers or not is a matter for conjecture. Harring and Myers seem to take the stand that the figure sent to them by Fadeew is identical with the species *D. haueri.* The differences listed above would readily separate this species from *D. rostratus. D. haueri* therefore remains as a distinct species from *D. rostratus* and *D. corystis.*

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EFFECTS OF SYNTHETIC THYROXINE AND GONADAL STEROIDS ON THE METABOLISM OF GOLDFISH[1]

WILLIAM S. HOAR

Abstract

Studies of both standard and active metabolism agree with most previous findings that thyroid hormone lacks a calorigenic effect while the gonadal steroids stimulate oxygen consumption. Thyroxine, testosterone, or stilboestrol will produce a marked increase in the excretion of nitrogen as measured by changes in the ammonia of the ambient water.

Introduction

Several workers have reported increased locomotor activity in fish treated with thyroid or gonadal hormones (12, 19). In this laboratory, synthetic thyroxine sodium, methyl testosterone, and stilboestrol (preparations of British Drug Houses used in concentrations of 1:2,500,000) have been shown to produce significant increases in the locomotor activity of young Pacific salmon and goldfish (13). The findings are not unexpected on the basis of numerous metabolism studies in mammals but this action of thyroxine in fish is puzzling since thyroid hormone has never been shown to produce a well-marked calorigenic effect in the poikilothermic vertebrates (12, 19). The most recent investigations agree that the thyroid hormone in fish produces no significant effect on its oxygen consumption (7, 16).

In this paper the possible calorigenic action of thyroxine is examined once more and data presented for the excretion of nitrogen by fish treated with synthetic thyroxine and gonadal steroids. Additional oxygen consumption studies were indicated for three reasons. In the first place, the most comprehensive investigation of this matter with respect to the goldfish (*Carassius auratus*) showed a definite calorigenic effect in fish treated with thyroid preparations or with thyrotropic hormone (18). Thus, the published results with goldfish are at variance with those on several other species of fish. In the second place, the specific synthetic hormone preparations used in the behavior studies (13) had produced marked effects on locomotor activity but had not been previously tested on the metabolism of fish. Finally, there is a suggestion in the literature that thyroid hormone might show effects on the metabolism of fish under extreme conditions of exercise (tissue oxygen supply restricted) which would not be apparent in resting animals (14). Both standard and active metabolism were, therefore, studied in fish.

Nitrogen excretion was measured in an attempt to explore a different aspect of metabolism and one which, in some vertebrates, has been shown to be affected by thyroid treatment. Indeed it was long ago appreciated that

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thyroid hormone did not regularly stimulate oxygen consumption in poikilotherms but would increase their nitrogen excretion—at least, in the case of the frog (15). Mansfeld (14) concluded "that the primary effect of thyroxin on cells consists in an accelerated splitting of protein". This matter seems not to have been examined in fishes or in poikilotherms other than the frog.

Materials and Methods

Goldfish were obtained from the Goldfish Supply Company, Stouffville, Ontario, and maintained under standard aquarium conditions at about 20° C. The fish weighed between 10 and 15 g. The standard diet was "Pablum" supplemented once or twice weekly with shrimp meal. The high protein diet was a dried mixture of 40% ground beef liver, 40% salmon viscera, and 20% shrimp meal.

The standard resting metabolism was measured at 20° C. in the open system described by Fry and Hart (8). The active metabolism was measured by placing individual goldfish in sealed quart bottles (about 900 ml. of water) for 1 hour and determining the change in oxygen content of the water by the Winkler method. In these tests of active metabolism, half of the fish were dipped directly from the aquaria and placed in the jars while the other half was first forced to swim for 15 minutes against a strong current of water created in the circular tubs previously used to study the rheotactic responses of young salmon (11). All tests were carried out at approximately 20° C.

Nitrogen excretion was measured as ammonia produced (sometimes also total nitrogen) in the ambient water by a definite weight of goldfish (usually about 100 g.) during a 24 hour period. The apparatus consisted of wide-mouthed Erlenmeyer flasks (capacity 2500 ml.) each fitted with a small glass nipple near the bottom and another near the top at a level which maintained the water in the flask at 2000 ml. The flasks were placed in a constant temperature bath (20° C.) and the fish acclimatized to the flasks in darkness for about 16 hours prior to the start of nitrogen collection. After this acclimatization period sufficient constant temperature water was circulated through the flasks to wash out all feces and produce a complete change of the solution. Flasks were then closed without disturbing the fish and left for 24 hours. A gentle flow of compressed air was maintained in each flask. The ambient water was analyzed at the end of 24 hours. Ammonia was determined by direct nesslerization (10). The total nitrogen was determined by nesslerization after micro-Kjeldahl digestion with concentrated sulphuric acid (10).

Values obtained in this way are considered to be a reliable index of relative amounts of nitrogen excreted by the different groups of fish. Unfortunately they provide no information as to the different nitrogenous constituents. Even the direct Nessler reaction may measure compounds other than ammonia (10) and this was evidently the case in the present study since treatment of ambient water samples with permutit did not remove all of the material responsible for the Nessler color reaction.

Fish were treated with synthetic thyroxine sodium, methyl testosterone, or stilboestrol (British Drug Houses preparations used in concentrations of 1:2,500,000) by immersing them in solutions of the drugs. The thyroxine is a *dl*-racemic mixture which complies with the specifications of the 1932 British Pharmacopoeia. Solutions were changed every third day.

Results

Oxygen Consumption

In duplicate experiments, the standard resting oxygen consumption was measured after 3, 7, 14, and 21 days of treatment with thyroxine or testosterone. In each experiment five oxygen analyses were made at approximately equal intervals during a 24 hour period. The values presented in Table I are averages for these five determinations. The calorigenic effect is evident in the testosterone but not in the thyroxine treated fish. The difference between the means of the control and the testosterone treated fish is statistically significant ($P < .01$) but this is not the case with the thyroxine treated fish ($P > .05$).

TABLE I

OXYGEN CONSUMPTION (ML./G./HOUR) FOR GOLDFISH MAINTAINED UNDER STANDARD CONDITIONS. VALUES ARE MEANS FOR FIVE DETERMINATIONS MADE AT INTERVALS DURING 24 HOUR TEST PERIOD AT 20° C. DATA BY R. R. GARDNER, JUNE TO AUGUST, 1953

Days treated	Experiment series	Control	Thyroxine	Testosterone
3	A	0.125	0.112	0.134
	B	0.111	0.116	0.149
7	A	0.087	0.102	0.125
	B	0.134	0.129	0.144
14	A	0.096	0.093	0.116
	B	0.139	0.117	0.135
21	A	0.094	0.089	0.133
	B	0.122	0.116	0.160
	Mean	0.114	0.109	0.137

Fish were also studied in a closed system after a period of activity. In half of the tests the fish were moved directly from the aquarium to the respiration chambers; in the other half the fish were first forced to swim for 15 minutes against a strong current. Comparative results for 280 fish are given in Table II and show no significant difference between the groups. Moving the fish directly from the aquarium to the bottles involved a brief struggle in air, which was evidently as effective in stimulating oxygen consumption as the 15 minute period of exercise. On the basis of this comparison, no distinction was made between the two groups in the analysis of hormone effects.

TABLE II

COMPARISON OF 140 "RESTING" AND 140 "EXERCISED" GOLDFISH IN CLOSED RESPIROMETERS. OXYGEN CONSUMPTION IN ML./G./HOUR, TEMPERATURE 20° C. DECEMBER 1953 TO JANUARY 1954

	Mean	Standard deviation	95% fiducial limits
"Resting"	0.267	0.012	0.210 to 0.293
"Exercised"	0.261	0.012	0.241 to 0.281

Oxygen consumption of groups of fish treated with the three synthetic hormones is given in Table III. The mean value for the oxygen consumption of 100 individual control goldfish was 0.240 ml./g./hour with 95% fiducial limits of 0.204 and 0.277. The mean values for testosterone and stilboestrol are significantly higher than the mean of the controls but the thyroxine value lies within the fiducial limits. As in the case of the measurements of standard metabolism, it is evident that thyroxine lacks a calorigenic effect while the gonadal steroids stimulate oxygen consumption.

TABLE III

MEAN OXYGEN CONSUMPTIONS (ML./G./HOUR) FOR 20 FISH TREATED WITH HORMONES AT 20° C. DECEMBER 1953 TO JANUARY 1954

Period of treatment, days	Thyroxine	Testosterone	Stilboestrol
7	0.259	0.262	0.271
14	0.225	0.343	0.314
21	0.266	0.265	0.285
Mean for all	0.250	0.290	0.290

Nitrogen Excretion

About 75% of the nitrogen excreted by the goldfish is ammonia and most of this (as well as the urea) is said to be excreted extrarenally through the gills (4, 20). It should be possible, then, to measure changes in protein metabolism by analyses for ammonia nitrogen in the ambient water. Analyses for total nitrogen in the ambient water would also serve as an index of nitrogen metabolism although this might be somewhat more variable since the urinary bladder will only empty periodically and such constituents as creatine, creatinine, and uric acid are excreted through the kidney (4). However, with several goldfish in the metabolism flasks and collections continued for 24 hours, reasonably consistent results are to be expected.

The data summarized in Fig. 1 show that thyroxine as well as the gonadal steroids produce a marked elevation in the ammonia nitrogen content of the ambient water of the goldfish. The values are presented as percentage variation from the values for a group of controls maintained without hormone

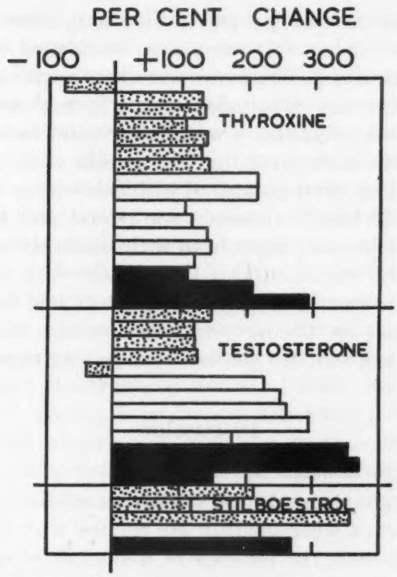

FIG. 1. Effect of thyroxine, testosterone, and stilboestrol on nitrogen excretion of goldfish expressed as per cent variation from control value. Stippled bars, 7 days' treatment; empty bars, 14 days' treatment; black bars, 21 days' treatment. All data are for winter fish.

treatment but otherwise under conditions identical with those of the experimentals. A control experiment was always carried out side by side with the hormone treated fish since several factors, such as diet, season, age (growth), and activity may be expected to affect the actual amounts of nitrogen excreted. Although the collection of excretion products was begun after a 16 to 20 hour period of starvation the effect of diet is still evident. Figures summarized in Table IV show that fish feeding heavily on a high protein diet may produce almost twice as much ammonia under these conditions.

TABLE IV

MEAN VALUES FOR AMMONIA EXCRETED BY GOLDFISH PREVIOUSLY FED A LOW PROTEIN (PABLUM) OR A HIGH PROTEIN (MEAT) DIET EXPRESSED AS MG. NITROGEN/KG. FISH/24 HOURS AND AS PER CENT OF TOTAL NITROGEN IN THE AMBIENT WATER. TEMPERATURE 20° C. SERIES A, WINTER 1955; SERIES B, WINTER, 1956. DATA BY AKI HORII

	Low protein			High protein		
Series	Number tests	Mg.	%	Number tests	Mg.	%
A	6	69.7	85.9	2	107.2	78.7
B	3	59.1	54.0	3	127.1	85.0

In the tests summarized in Fig. 1 precautions were taken to avoid disturbing the fish from the time when they were first introduced into the flasks until the end of the experiment. The flasks were kept under cover in continuous darkness and all necessary manipulations performed without opening this system to light. Such precautions were later found to be unnecessary. In duplicate experiments, each involving four groups of fish, the usual controls (maintained in darkness) were compared with fish kept in the light and greatly disturbed after the 16 hour acclimatization period and before nitrogen collection was begun by pouring them from their flasks into a bucket, capturing them in the hand, and then returning them to the flask. In one test the ammonia nitrogen value was 2% higher for the exercised fish and in the other test it was 9% higher for the non-exercised group. Such variation is well within the limits of error in this method of collecting nitrogen from groups of fish.

Discussion

Metabolic Effects of the Gonadal Steroids

The elevated oxygen consumption is consistent with the stimulated locomotor activity observed when goldfish are treated with the gonadal steroids. It is also consistent with the findings in mammals where metabolism may increase 5 to 15% during treatment with androgen (6). The mechanism, however, is not clear and the matter is one of considerable interest to the endocrinologist interested in behavior. Two possibilities are obvious. The steroids may increase in some way the reactivity of neuromuscular mechanisms thus promoting the locomotor activity which indirectly results in an increased demand for oxygen. On the other hand, these steroids might act generally throughout the body stimulating oxidative metabolism in a variety of tissues. Although *in vitro* studies have been carried out, the results do not permit definite conclusions. In some cases steroids have been shown to increase and in others to decrease cellular metabolism and the effect appears to depend markedly on the concentration of steroid (3, 9).

The steroid effects on nitrogen metabolism of the goldfish were opposite to those usually found in warm-blooded animals. In the mammals, androgens and estrogens promote protein anabolism and nitrogen retention (2, 5, 6, 24). Increased growth of muscle and proliferation of the tissues of the reproductive system are characteristic. Effects of the gonadal steroids on the nitrogen excretion of fish seem not to have been investigated. In the goldfish, sexual maturation is marked by very few changes in accessory reproductive structures and the growth rate in general is slow. It seems likely that the observed results are in some way related to the stimulated locomotor activity rather than effects on growing tissues.

The significance of the ammonia excreted by the fresh-water teleost fish is not clear. Baldwin (1) has argued that the ammonia excretion represents a primitive means of removing nitrogenous wastes and is found in fresh-water aquatic organisms where large quantities of water are available to wash

away this extremely toxic product. Where the tissue water is restricted, as is the case in marine teleosts or terrestrial vertebrates, the ammonia is converted to less toxic compounds such as urea or uric acid. Smith (23), on the other hand, argues that urea is the nitrogenous excretion product in fresh-water fish as in most other vertebrates and that the ammonia is formed secondarily from glutamine (25) in relation to the acid–base balance of the fish. In this case, increased activity would increase the demands for ammonia to neutralize the acid products of metabolism. Smith bases his argument primarily on studies of the lungfish where urea is the product formed during the prolonged inactivity of estivation (22, 23). In connection with Smith's theory it may be noted that in the present study the percentage of ammonia in the total nitrogen of the ambient water was not increased by exercising the fish in a manner known to produce a sharp rise in oxygen consumption.

On the basis of either Baldwin's or Smith's theory one might expect an elevated production of ammonia in fasting fish known to show increased locomotor activity. Fish receiving no food may be expected to call on their nitrogen reserves if the metabolic demands are high; if ammonia is required to neutralize acid metabolites an increased output in the more active fish is likewise expected.

Metabolic Effects of Thyroxine

In line with most previous findings, thyroxine does not stimulate oxidative metabolism in the goldfish. Nitrogen excretion, however, is considerably elevated indicating that this hormone is stimulating some aspect of tissue metabolism. As previously pointed out a similar effect has been observed in measurements of the nitrogen excretion of frogs (15).

Failure to find a calorigenic effect of thyroxine is baffling in view of the increased locomotor activity which follows thyroxine treatment even though the locomotor stimulation is usually less than that observed with the gonadal steroids (13). Nitrogen excretion is likewise lower in the thyroxine treated animals but definitely higher than the controls.

An explanation of these results will probably depend on a demonstration of the function of the thyroid hormone at the cellular level of organization. As yet no satisfactory explanation of the role of this essential material has been possible.

Protein anabolic as well as protein catabolic effects have been observed in both poikilotherms and homoiotherms treated with thyroid preparations. In fish, under certain conditions, thyroid hormone seems to stimulate growth, metamorphosis, guanine deposition, and epidermal thickening (12, 16, 19). In the frog, where oxidative metabolism shows little or no response to thyroid treatment an increased nitrogen excretion is evident. On the basis of these and of a great many *in vitro* studies, Mansfeld (14) suggested that the primary effect of this hormone is one of accelerating the splitting of protein and that the products so produced lead secondarily to increased oxygen consumption. In cold-blooded animals, he considered the concentration of breakdown products insufficient or reached too slowly to call forth the stimulating effect on

oxidation processes. Pickford (19), after a careful survey of the pertinent information, concludes that "in spite of the incomplete nature of the evidence, it appears that the thyroid hormone has parallel effects in cold- and warm-blooded vertebrates, but that in the former, low temperature and cold adaptation tend to mask the response". In mammals, both anabolic and catabolic effects have been repeatedly described (2, 6, 21, 24) and one writer suggests that the hormone acts in maintaining an equilibrium between protein anabolism and protein catabolism (17).

In summary, the data show that both thyroxine and the gonadal steroids stimulate the metabolism of goldfish. That they may have a very different action on metabolism, however, is indicated by the fact that only the steroids affect oxygen consumption while both steroids and thyroxine increase nitrogen excretion. These experiments provide no evidence concerning the mechanisms involved nor do they permit a decision as to the point of action of the materials—whether directly through the central nervous system or by a generalized stimulation of the metabolism of tissues throughout the body.

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BORING SPONGES, CLIONA SPECIES, OF EASTERN CANADA, WITH A NOTE ON THE VALIDITY OF C. LOBATA[1]

FREDERICK E. WARBURTON[2]

Abstract

Cliona celata occurs in Prince Edward Island; *C. lobata* in Prince Edward Island and on the Gulf shore of New Brunswick; *C. vastifica* in the Bay of Fundy, off Sable Island, off Prince Edward Island, and off Newfoundland. Intraspecies grafts of *C. celata* and *C. lobata* succeeded, but interspecies grafts failed. This should remove doubt about the taxonomic distinctness of these two species.

Introduction

The common boring sponge (*Cliona celata*) was reported from the north shore of Prince Edward Island by Lambe (3) in 1896. Since then little that is new has been published on the occurrence of the genus in eastern Canadian waters. In discussions of the oyster fishery, a few writers have mentioned *C. celata* in Malpeque Bay, P.E.I. (Stafford, 1913 (8); Needler, 1941 (6)), and the only Canadian material used by Old (7) in his 1941 review of the Atlantic North American species was *C. celata* collected by Needler from Malpeque Bay.

During an investigation of the biology of boring sponges carried on by the Fisheries Research Board of Canada from its Biological Sub-Station, Ellerslie, P.E.I., two other species were found in various localities. Collections were made by A. A. Skinner, L. M. Dickie, J. C. Medcof, and the writer. Identification of species was based on spiculation, using the key prepared by Old (1941 (7)).

Distribution

Cliona celata Grant (1826 (1))

This is the commonest species in Malpeque Bay, and occurs frequently on other parts of the north shore of Prince Edward Island, often well up in estuaries. It has been found in the shells of living and dead oysters (*Crassostrea virginica*), mussels (*Mytilus edulis*), slipper limpets (*Crepidula fornicata*), and barnacles (*Balanus* sp.), and in empty shells of bar clams (*Spisula solidissima*), quahaugs (*Venus mercenaria*), and soft-shelled clams (*Mya arenaria*), as well as in limestone pebbles. Probably it will inhabit any type of shell or other calcareous substance available in its environment.

Cliona vastifica Hancock (1849 (2))

This species is common in scallops (*Placopecten magellanicus*) off Digby, N.S., at St. Andrews, N.B., and off Sable Island. It also occurs in empty

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[2]Author's present address: c/o Department of Anatomy, McGill University, Montreal, Que.

conch shells (*Neptunea* sp.) at Digby, and in conchs and periwinkles (*Littorina littorea*), washed ashore on the north shore of Prince Edward Island. One specimen, in an Iceland scallop shell (*Chlamys islandicus*), was obtained from Hare Bay, Nfld.

Cliona lobata Hancock (1849 (2))

This sponge was found in the shells of oysters from the Miramichi area and from Shippegan, N.B., and in oysters from Malpeque Bay, P.E.I. However, in Prince Edward Island, it is less common than *C. celata*. A specimen from Malpeque Bay had bored in a *Polinices* shell occupied by a hermit crab.

Taxonomic Validity of *C. lobata*

De Laubenfels (1949 (5)) mentions that many authors, following Topsent (1900 (9)), consider *C. lobata* synonymous with *C. celata*, since it has been reported that young specimens of *celata* contain spirasters, which are one of the diagnostic features of *lobata* given by Hancock (1849 (2)), and used as a key character by Old (1941 (7)), who maintains the distinction between the two species. The writer has never found spirasters in *celata* at any size, but found them consistently numerous in all specimens of *lobata*.

Wilson (1911 (10)) found that cells of *Microciona prolifera*, dissociated by being pressed through bolting silk, would reconstitute themselves into miniature sponges, but that cells of *Lissodendoryx* added to the suspension formed separate reconstitution bodies. De Laubenfels (1927 (4)) tried all possible bispecific mixtures of cells of three species of *Pachychalina* forced through bolting silk; none of the mixtures formed functional sponges, although dissociated cells of individual species metamorphosed successfully.

This physiological approach would seem to be a useful accessory to the usual methods of sponge taxonomy. The writer has adapted and simplified it to test the debated distinction between *C. celata* and *C. lobata*. Fragments of *C. celata* were taken from different shells and piled together in a dish of sea water. These became grafted together, and within a few days the fused mass remodelled itself into a single sponge, with a functional canal system. Fragments of *C. lobata* behaved in the same way. However, it proved impossible to graft fragments of *C. lobata* to fragments of *C. celata*. When fragments of the two types were piled together, whichever formed the majority of the mass remodelled itself into a functional sponge, but the fragments of the other species remained separate as rounded, structureless masses. The writer believes that these experiments are strong evidence for the validity of the specific distinction between *C. celata* and *C. lobata*.

Acknowledgments

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VARIATION IN NUMBER OF DORSAL SPINES IN THE BROOK STICKLEBACK, EUCALIA INCONSTANS[1]

G. H. LAWLER

Abstract

The brook stickleback, *Eucalia inconstans* (Kirtland), is usually described as possessing five or six dorsal spines and the species is commonly called the five-spine stickleback. In Heming Lake, Manitoba, fish with six dorsal spines predominate. The occurrence of sticklebacks with a high number of dorsal spines is noted from other Manitoba lakes and to a lesser extent in samples from some Ontario lakes. Indications are that the dorsal spines are more numerous in fish from the Hudson Bay drainage than from the Great Lakes region. An increase in the number of dorsal spines with increasing latitude is apparent.

Introduction

During the course of an investigation of the fishes of Heming Lake, Manitoba, it was observed that the brook stickleback, *Eucalia inconstans* (Kirtland), had an unusually high average number of dorsal spines. Hubbs and Lagler (2) described *Eucalia inconstans* as possessing five or six spines while Dymond (1) stated that usually five spines are present. No records of spine counts from different geographical areas were found and consequently specimens from several localities in Manitoba and Ontario were examined to determine whether the variation observed in the Heming Lake sticklebacks was also characteristic of these other areas.

Materials and Discussion

Dorsal spine counts were made on 140 specimens of *Eucalia inconstans* from Heming Lake, ranging in size from 39 to 76 mm. total length. Of those examined, 66% had six dorsal spines, 31% had five spines, and 3% had seven spines (Table I). No sticklebacks were found that had only four dorsal spines.

Counts made on 202 specimens of *Eucalia inconstans* representative of various parts of Manitoba are shown in Table I. The ratio of dorsal spines was four-spine—7; five-spine—156; six-spine—39. The number of six-spined fish was considerably higher than those with four spines and in one sample they constituted 54% of the sample. In other samples those sticklebacks with six spines made up 37, 36, and 25% of the samples, respectively. Of 12 samples of Manitoba sticklebacks examined only two did not contain fish that possessed six dorsal spines.

It was considered that possibly the higher numbers of dorsal spines in *Eucalia inconstans* were more prevalent in Manitoba so the scope of the survey was broadened to include the Province of Ontario. Samples from several Ontario localities ranging from the St. Lawrence River region to

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TABLE I

VARIATION IN NUMBER OF DORSAL SPINES IN *Eucalia inconstans* FROM SEVERAL REGIONS OF MANITOBA AND ONTARIO, CANADA, ARRANGED IN ORDER FROM SOUTH TO NORTH

Locality	Lat.	Long.	IV	V	VI	VII	Percentage with six spines	No. on map
Manitoba								
Long River	49° 8′	99° 00′	0	7	4		36	1
Pembina River	49° 13′	99° 42′	0	3	0		0	17
Mary Jane Creek	49° 13′	98° 43′	0	10	2		16	0
Hanson's Creek	49° 45′	95° 15′	0	6	2		25	2
Rennie Creek	49° 45′	95° 35′	0	24	2		8	5
LaSalle River	49° 54′	97° 46′	0	17	10		37	6
Cook's Creek	50° 00′	98° 5′	0	9	1		10	3
Marsh Lake	50° 15′	97° 15′	1	4	1		16	4
Clear Lake	50° 41′	100° 00′	4	54	11		16	7
Branch of Vermillion River	51° 10′	100° 3′	2	17	0		0	8
South Pine River	51° 47′	100° 20′	0	5	6		54	9
Heming Lake	54° 53′	101° 7′		43	92	5	66	10
Ontario								
Pickering Twp. near Audley	43° 55′	79° 00′	1	50	6		11	11
Housic Creek	45° 00′	75° 15′	1	12	1		7	12
Cache Lake	45° 32′	78° 35′	5	38	2		4	13
Cedar Lake	49° 20′	88° 10′	2	14	3		16	14
Fairloch Lake	49° 30′	88° 00′	4	19	0		0	15
Bog Lake near Fort Albany	52°	81°	1	17	12		40	16

James Bay were examined and spine counts recorded (Table I). The percentage of sticklebacks with six spines ranged from 7 to 40 and only one sample did not contain fish with six dorsal spines. Only the northern sample from the James Bay region had an unusually high proportion of six-spined fish. The percentage of *Eucalia inconstans* from Ontario waters with six spines was slightly lower than in Manitoba.

The percentage of the samples containing six-spined sticklebacks from Manitoba and Ontario were plotted on a map (Fig. 1) to see if a distributional pattern was evident. The limited distributional records indicate that the dorsal spines of *Eucalia inconstans* are more numerous in fish found in the Hudson Bay drainage than in the Great Lakes region. Also it would appear that there is an increase in the number of dorsal spines with increasing latitude as the northernmost fish had the greatest number of dorsal spines. The latter statement tends to agree with the findings of Walters (4) whose data on the nine-spine stickleback, *Pungitius pungitius*, revealed a tendency, in ranging from colder to warmer climates, for lower spine counts to appear, and higher spine counts to disappear, accompanied by a downward shift in the mode and mean. He states "it appears as though some factor in the environment

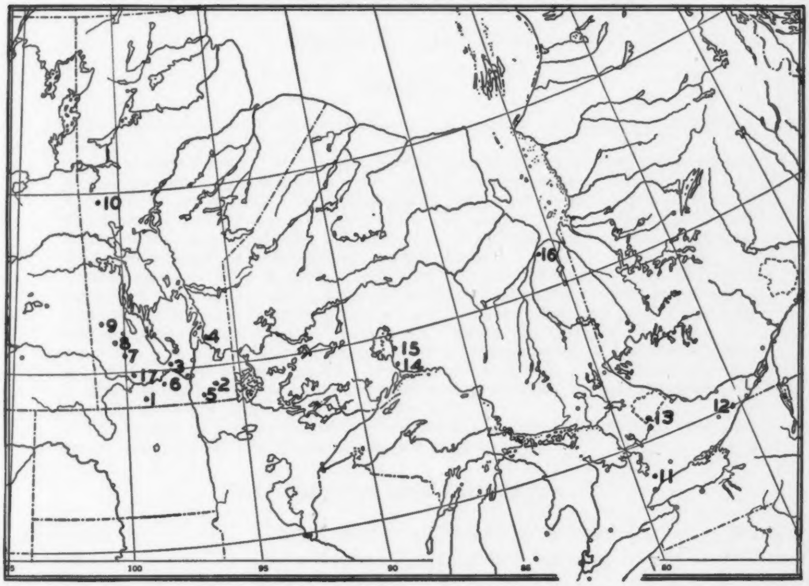

Fig. 1. Outline map of Manitoba and Ontario showing localities of collections of *Eucalia inconstans.*

were selectively eliminating those individuals with spine counts not adaptive to the locality", which possibly explains why no sticklebacks with four spines were collected at Heming Lake. Further evidence of latitudinal variation in spine counts is shown by Vladykov (3).

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NUCLEAR ACTIVITY OF BLOOD CELLS[1]

VIBEKE E. ENGELBERT

Abstract

This paper presents the continuation of studies on blood cell behaviors published earlier by Engelbert.

It was then shown that "nuclear bodies" named "basic nuclear units" passed through several stages of a nuclear life history which was believed to hold the key to blood cell cytogenesis. Blood cell polymorphism or pleomorphism is believed to be products of the many staged nuclear life history. The nuclear life history can be experimentally altered, for example by foreign proteins. The basic nuclear units are believed to be reproductive organelles.

Additional evidence of nuclear behaviors fitting into the nuclear life history is presented. Formation and release of vesicles by myeloblast nuclei was demonstrated earlier. Evidence is presented here to show that the production and release of these intranuclear vesicles is not a sign of degeneration, but a process by which new cells can be produced through reassociation of released nuclear materials. Some released vesicles become red blood cells. Extreme variations in shape of myeloblast nuclei are not signs of degeneration, but are characteristic nuclear behaviors associated with nuclear activity.

The release of new small nuclei, intranuclear vesicles, and various granules are nuclear activities leading to formation of new cells.

Introduction and Literature

Cytogenesis as well as the function of blood cells have for years been of interest to workers in many fields. The author showed in 1953 (10) that lymphocytes released substances through the formation of tubular structures and suggested that the process of release was not a breakdown process but the normal behavior of lymphocytes. A further report on behavior of lymphocytes was made in 1956 (11). The following represents a brief outline of that report. It was shown that both lymphocytes and myeloblasts released nuclear bodies by means of tubular structures. The tubes were produced either from the nuclear membrane alone or in association with the peripheral chromatin. Two separate groups of chromatin were described for the myeloblasts. The first is a peripheral group which lies close to the nuclear membrane, forming a homogeneous almost shell-like layer, and is identical with the nucleus of the classical myeloblast. The second group of chromatin lies in the center of the nucleus and is hidden by the shell-like peripheral chromatin. The peripheral chromatin may reorganize into granular bodies that come to lie in the nucleoplasm. With the disappearance of the homogeneous shell-like layer, the central group of chromatin becomes visible. The central group can at this time show the many lobed nuclear structure familiar from the heterophilic leucocyte (neutrophilic in man).

The central group of chromatin may also give rise to several small nuclei that are released individually and finally become new small lymphocytes.

Under influence of foreign serum the chromatin of myeloblasts does not form the peripheral shell-like layer (classical nucleus), but the chromatin passes through divisions and rearrangements that resemble cytogamy in

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Contribution from Department of Zoology, University of Toronto, Toronto, Ontario.

Paramecium. Eventually small nuclear bodies project into cone-like extensions of the nuclear membrane. Each cone becomes a tube and finally the nuclear bodies are released with a layer of nuclear membrane around them. During the divisions and rearrangements mentioned above, the nucleus extends and contracts.

Downey and Weidenreich (8) showed already in 1912 that lymphocytes released pieces of their cytoplasm. Weill (20) and Williamson (22) demonstrated similar phenomena. Nuclear fragments of lymphocytes were shown in culture preparations by Popoff (16), Tschassownikow (17), and Emmart (9). One of Popoff's illustrations is shown in Fig. 13-3 in the textbook of histology by Maximow and Bloom (15). Many "nuclear fragments" can be seen between the lymphocytes. Fig. 13-2 in the same book is a section from human thymus. Here the nuclei of the lymphocytes have been caught in different stages of extension and contraction. In the upper left hand corner near the margin of the Hassall's body, a lymphocyte is extruding three small nuclear bodies. The "nuclear fragments" and nuclear bodies mentioned above are believed by the author to represent the basic nuclear units that are released as a normal phase of nuclear life history.

The characteristic lymphocytic behavior of releasing substances have in recent years been called "cytoplasmic budding" by White (21) and Frank and Dougherty (12). White states "The term dissolution of lymphocytes has been used in the sense of a disintegration characterized by karyorrhexis, pycnosis, or a shedding of the cytoplasm of the cell. This shedding or budding of lymphocyte cytoplasm was shown by Downey and Weidenreich to be a normal property of these cells. All of the mentioned cellular alterations may be seen in the lymphoid tissues at the time of dissolution of lymphocytes". The three workers mentioned above consider the cytoplasmic budding the beginning of a destructive process resulting in pycnosis and karyorrhexis, and dissolution of lymphocytes. In a paper published in 1882 by Watney (19) on the thymus one illustration clearly shows two cells with fairly long tubes. Watney did neither label nor describe the two cells, but obviously illustrated carefully what he saw.

Blair (3), who worked in this laboratory, observed 5590 normal lymphocytes from thymi of mammals and chick embryos. She counted 4591 cells that showed one or more tubes; 551 nuclear bodies were found inside tubes.

In order to observe the tubes, the peripheral and central chromatin, and other phenomena mentioned above, one must make imprint preparations. Both the smear method as well as sectioning techniques destroy the critical stages. Further, in order to observe the various and complex stages of nuclear activity, fixation must be fast, as the nucleus may contract and, therefore, not disclose the phenomena that can be seen in the living nucleus.

It was shown (Engelbert (11)) that granulocytes as well as lymphocytes were produced in the spleen. The term myeloblast was used for the "blast cell" in the spleen, because its morphology in certain stages appeared identical with the classical myeloblast. Also its behavior when in motion fitted the

form of movement exhibited by the myeloblasts in cultures *in vitro,* which was shown by Lewis (14) to be in a spiralling fashion. It would probably be better to call the "splenic blast cell" a hemocytoblast instead of a myeloblast. The term myeloblast is, however, being kept in this paper because the writer feels that a change of terms at this time would only lead to confusion. Further, the writer believes that eventually it will be found that similar "blast cells" or "blood mother cells" exist in all the blood forming organs. Until such time, no new terms should be added; rather, efforts should be made to determine how this similarity is expressed and what conditions (foreign proteins, hormones, etc.) can cause variance and dissimilarities in the behavior and morphology of the "blood mother cells" or "blast cells".

Material and Methods*

Materials

The spleens of mammals have as before provided the material for the study of blood cells, but livers of young animals were also used in the present study.

Isolated nuclei from calf thymus and liver and prepared by Dounce's method (7) in the Department of Biochemistry were put into a culture medium and observed for a week and longer.

Methods

Imprints of cut surfaces of spleens and livers were fixed quickly in methyl alcohol or iodine vapor and stained with the May-Gründwald plus Giemsa stain.

Smear preparations were made of the isolated nuclei of calf thymus and liver. The smears were fixed in methyl alcohol and stained in May-Gründwald plus Giemsa stain. Small samples of isolated nuclei were also added to a medium consisting of 1 part normal horse serum, 1 part Tyrode's solution (Earl's modification) on an ordinary slide covered with a 22 × 40 mm. cover slip of 0 thickness, then sealed with hot paraffin.

Nuclear Activities in Myeloblasts

Morphological Changes and Formation of Intranuclear Vesicles

Contractions and extensions of nuclei can occur quickly, as explained earlier (Engelbert (11)). During these nuclear movements, intranuclear vesicles, parts of nucleoplasm, or new small nuclei (basic nuclear units) can be released to the surrounding medium. These behaviors, the writer believes, are normal and not pathological signs of breakdown (11, Figs. 7, 8, 9, 14, and 15).

In practically every imprint of normal spleen from different mammals (mice, rats, rabbits, hamsters, and arctic lemmings), the nuclei of myeloblasts can be seen to range (Fig. 1) from rounded, (*ma*), to elongated forms (ma_1, ma_2). As the nuclei elongate they possess very little, if any, cytoplasm. Finally the nuclei can become so stretched (ma_4), that they appear as long dark fibers.

* For further details, see earlier papers (Engelbert (10, 11)).

During the process of elongation and stretching, the nuclei release most of their nucleoplasm (Fig. 1, *rpl*). The nucleoplasm, when released, gives the typical appearance of "vacuolated cytoplasm" or "degenerative cytoplasm" (Dacie and White (5, Pl. IV, Figs. 2 and 4)), but the vacuoles are really small vesicles (Fig. 1, *rpl*). The vesicles fuse (Fig. 1, *ve*), thus forming larger vesicular structures (Fig. 1, ve_1). The vesicles may contain granules (11, Fig. 14), rods (Fig. 1, ve_1), or just a semifluid substance, with faint staining qualities.

Small granules that stain a pale bluish color with the May-Gründwald plus Giemsa stain usually surround the vesicles (Fig. 1, *rpl, ve*). (See also (11, Fig. 14).) The vesicles eventually become cellular elements (Fig. 1*a*, *vec*) that at first stain faintly. Many different vesicular stages can be clearly seen in Fig. 1*a*.

The stretched nuclei (Fig. 1, ma_4) that have released their nucleoplasm may contract and "curl up," thus once more forming a rounded nucleus (Figs. 2 and 3). The chromatin in these "curled up" nuclei is of a more dense and homogeneous consistency than the chromatin in the original rounded nuclei (Fig. 1, *ma*).

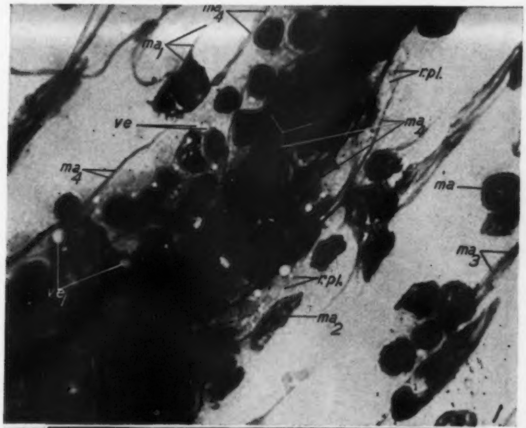
Release of Nuclear Bodies (Basic Nuclear Units) from Myeloblasts

Release of nuclear bodies, termed basic nuclear units, from myeloblasts and lymphocytes was described earlier (11). At that time it was also pointed out that the basic nuclear units could become organized into two nuclear groups, one just below the nuclear membrane, forming "the peripheral chromatin," the other, enclosed by the first, forming "the central chromatin." The central chromatin gives rise to new, small nuclei that can be released through a tubular structure.

A further example of the phenomenon just mentioned is presented in Figs. 4 and 5. These two figures are from an imprint of the liver of a 6-week-old rabbit. A very long tube (see arrows) extends from a nucleus (*ma*) lying on the right. Inside the tube near the end lies a nuclear body (*mi*) that soon will be released.

Other nuclear bodies (*mi*) can be seen in the tube. These nuclear bodies, the writer believes, are basic nuclear units. A nuclear body outside the tube, but adhering to a red blood cell (mi_1), and another nuclear body lying free (mi_2), can be seen also. Fig. 6, which is a photomicrograph of a 10μ scale, should be used in conjunction with Fig. 5. The similarity of the nuclear body (mi_1) to a Howell-Jolly body, as shown by Diggs (6, Pl. XXI, H and K), should be noted.

The number of nuclear bodies or basic nuclear units that can be produced by a group of central chromatin is not known at present. It is, however, possible both in fixed stained culture preparations, as well as in imprints, to find nuclei where the peripheral chromatin has been cracked open and one can then count a dozen or more nuclear bodies inside.



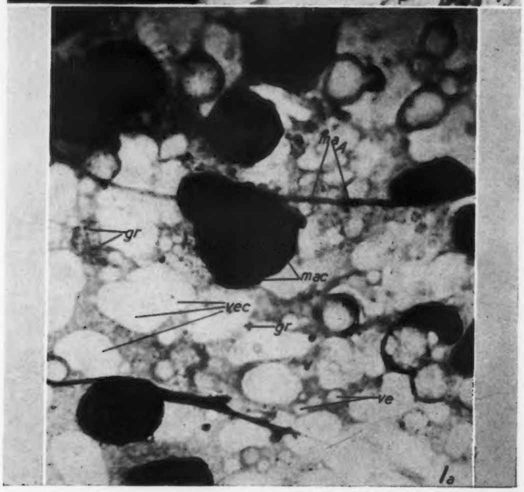


FIG. 1. Myeloblast nuclei, rounded form, with a rim of cytoplasm (*ma*) nuclei showing varying degrees of elongation (ma_1–ma_3) nuclei so stretched that they appear fibrous (ma_4). Spleen, normal ♀ mouse, 5 weeks old.

rpl—vesicles and granules ("vacuolated cytoplasm"); *ve*—two vesicles fusing; ve_1—vesicles containing rod-like bodies.

Photomicrographs. Zetopan, Reichert. Phase microscope oil immersion objective 100×, ocular 10× used for Figs. 8–12 inclusive.

Leitz ortholux microscope, used for all other figures. Objective 45×, ocular 10× used for Figs. 1 and 4; oil immersion objective 90×, ocular 10× used for rest of figures.

Leica camera with Micro Ibso attachment.

Kodak Micro-File film used for all photographs.

Ac. A—acetic alcohol 1:3.

Meth.—methanol. Iv.—Iodine vapor.

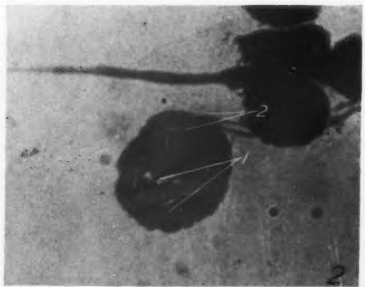
M.G. + G.—May-Gründwald plus Giemsa stain.

Feulg.—Feulgen nucleal stain.

FIG. 1*a*. Cell regeneration from vesicles and granules originally released by myeloblast nuclei. Spleen normal rabbit. Biopsy sample. Iv. M.G. + G.

ma_4—stretched myeloblast nucleus; *mac*—curled up myeloblast nucleus; *ve*—vesicle; *vec*—faintly staining cells and nuclei formed from vesicles; *gr*—granules.

PLATE II



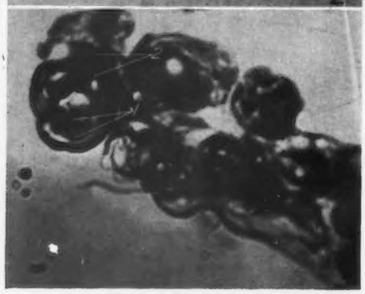
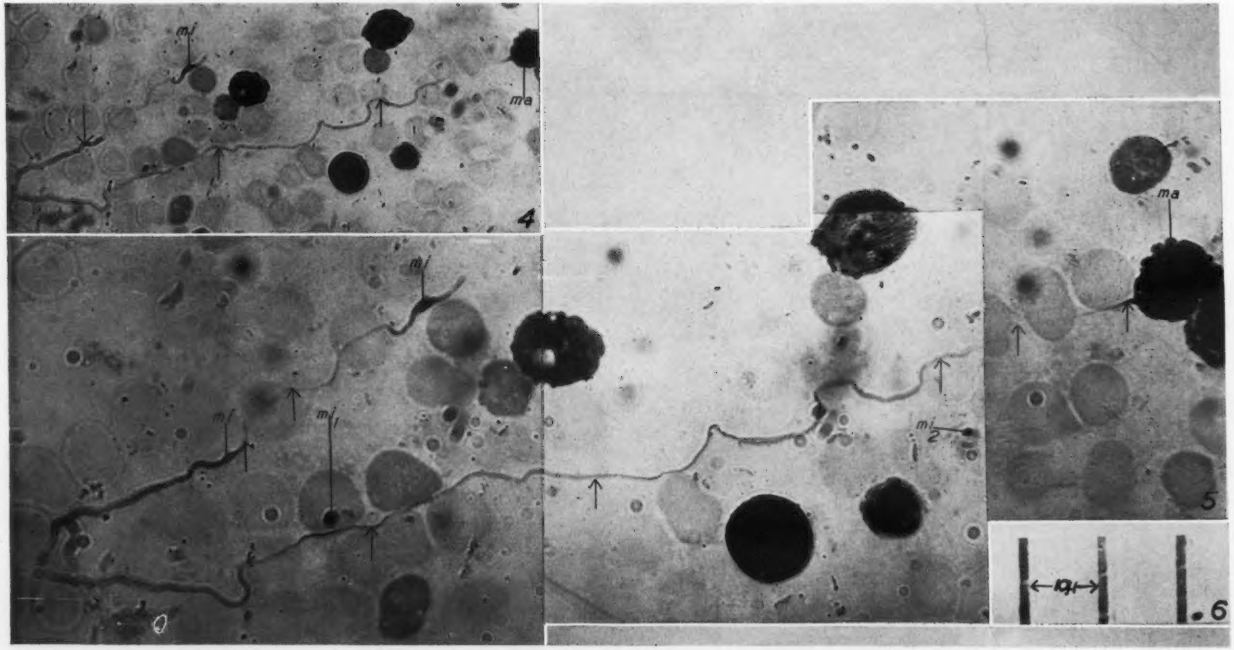


FIG. 2. Nucleus previously very much extended now curled up in a "watch-spring fashion"; outer portion (2) is curled around inner portion (1). Spleen, normal ♀ mouse 6 weeks old. Meth. M.G. + G.

FIG. 3. Stretched nuclei in the process of curling up, inner portion (1), outer portion (2). Spleen, ♀ mouse AKR strain, 9 weeks old. Meth. M.G. + G.

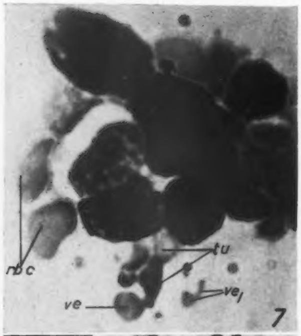


FIGS. 4 and 5. Myeloblast nucleus (*ma*) with long tube containing basic nuclear units (*mi*) that soon will become free. Arrows point to tube. Liver imprint, normal ♀ rabbit 6 weeks old. Meth. M.G. + G.

mi_1—basic nuclear unit adhering to a red blood cell; mi_2—basic nuclear unit lying free of other cells.

FIG. 6. The 10μ scale to be used for all figures except Figs. 1 and 4.

PLATE IV



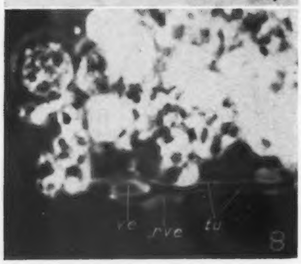
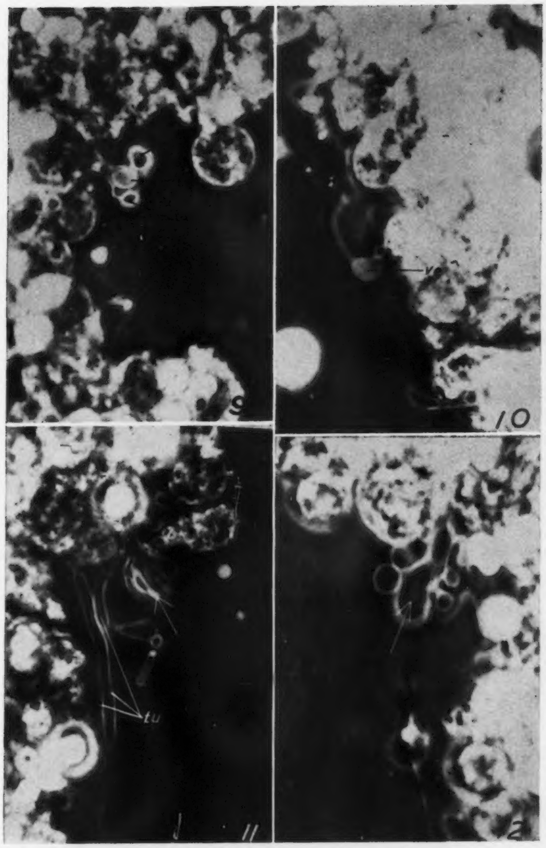


FIG. 7. Myeloblasts (*ma*) forming tubes (*tu*) and vesicle (*ve*). Spleen, normal ♀ mouse 6 weeks old. Meth. M.G. + G.
ve_1—newly released vesicle; *rbc*—erythrocytes.

FIG. 8. Isolated nuclei from calf liver cultured *in vitro* for 10 days.
rve—released vesicle; *tu*—tube; *ve*—vesicle.



FIGS. 9–12. Isolated nuclei from calf liver cultured *in vitro*.
FIGS. 10, 11, and 12 and Fig. 9 were photographed when the culture was respectively 9, 10, and 11 days old. Arrows point to vesicles that during the photography exhibited a gently undulating movement which caused their outer borders to become slightly blurred.
tu—tube; *ve*—vesicle.

Intranuclear Vesicles with Semifluid Contents

Release of intranuclear vesicles by myeloblasts may well prove to be a nuclear activity fully as important as release of nuclear bodies. Many vesicles contain a semifluid substance and present a very plastic and soft outer membrane. These vesicles, when first becoming free, will, in fixed imprints, often show rather angular outlines because of their soft surfaces (Fig. 7, ve_1). The vesicles stain a color that is identical with the color shown by young erythrocytes in May-Gründwald plus Giemsa preparations.

In Fig. 7, several large nuclei of myeloblasts are present. Tubular (*tu*) and vesicular (*ve*) structures extend out from two of these nuclei. Nearby lies a newly released vesicle (ve_1) and to the left are two young erythrocytes. Intranuclear vesicles staining in the same manner as red blood cells can be seen in a great number of cases inside the nuclei of myeloblasts. Stages such as shown in Fig. 7 are harder to find, because they seem easily damaged in the preparation.

Bonnichsen *et al.* (4) showed, after ultraviolet absorption microspectroscopy and cytochemical staining procedures, that certain nuclei are primarily the site of hemoglobin synthesis. The observations on behavior and morphology of nuclei presented in the present paper gives support to this work.

Further proof that formation of tubes and vesicles is a nuclear activity is presented in Figs. 8–12 inclusive. These figures show isolated nuclei from calf liver, kept in a medium of normal horse serum and Tyrode's solution 1:1 for 11 days. After 6 days the nuclei began to form tubes and vesicles. A gentle undulating movement of the outer membranes of vesicles was often noticed. This movement caused a slight blurring of the outer membranes during time exposures as can be seen in Figs. 11 and 12.

Nuclear Activities and Nuclear Life History

The nuclear activities that have been dealt with in this and earlier papers, namely release of nuclear bodies, intranuclear vesicles, and granules, are believed not to be a breakdown process leading to cell death, but a part of nuclear life history.

The behavior of nuclear bodies, which involves phases from associations in large nuclei to small individual free nuclei or to small granules as small as chromomeres, may on the surface look like a breakdown process.

Since the discovery of the nucleus by Brown in 1835, research has centered around its role in cell division. Mitosis is a process that because of its distinct phases and its relatively slow procedure can be easily observed.

The behaviors reported earlier as well as in the present paper can happen either very rapidly as, for example, contraction of the nucleus, release of nuclear bodies, release of vesicles: all of which can happen in seconds or less and can easily be missed by the observer. Other events proceed slowly, e.g. the fusion of vesicles and the reorganization of nuclear bodies (basic nuclear units) into new nuclear combinations. These differences in speed

no doubt have played a role in making certain nuclear events more obvious than others and so gained the attention of research workers more readily.

A cyclic life history for lymphocytes was suggested last year (Engelbert (11)). Recent work by Trowell (18) on "re-utilization of lymphocytes in lymphopoiesis" supports the idea of a cyclic life history of the nucleic acids of lymphocytes. Trowell believes, however, that a process of phagocytosis of pycnotic lymphocytes is an important link in the cycle.

The writer does not believe phagocytosis or pycnosis constitutes a main link in the cycle, but that nuclear behaviors such as outlined earlier (11), as well as in the present paper, hold the key to lymphogenesis and blood cell cytogenesis in general.

Discussion

The classical myeloblast is described as a rounded cell with a rim of cytoplasm. The myeloblast of the spleen shows this rounded stage as well as other more or less elongated stages (Fig. 1).

The myeloblast nucleus can contract and extend itself (Engelbert (11, Fig. 14)) and the time that elapses between these stages is relatively short. Contraction of the nucleus in large lymphoid cells (p. 721) also takes place and can proceed very fast, thus squeezing out some nucleoplasm, which forms a rim (cytoplasm) around the contracted nucleus.

The classical myeloblast, with the rounded nucleus and a rim of cytoplasm, the author believes to be a partially contracted stage. The elongated stages of myeloblasts, which generally in the literature are called "degenerative", were shown to produce anlagen of new cells (Figs. 7, 8, and 9). There does not seem to be any critical evidence in the literature to substantiate the term "degeneration" for the elongated stages of the myeloblast. These stages appear not to have been studied in detail. The seemingly "vacuolated cytoplasm" often seen around myeloblasts have also been called "degenerative". It may be that because pathological tissues often have revealed odd-shaped nuclei or "vacuolated" cytoplasm (see, for example, Dacie and White (5)) that similar structures in normal tissues have been considered a sign of some form of breakdown. Very densely staining nuclei are often called "pycnotic", suggesting that the chromatin of the dense nucleus is not normal. A contracted nucleus stains much darker than a more expanded nucleus and we should know more about the behavior of nuclei before we consider any "dense" nucleus to be degenerative.

Leuchtenberger (13) studied pycnotic nuclear degeneration with cytochemical methods. She showed that during different stages of pycnosis the desoxyribonucleic acid (DNA) lost its stainability with methyl green to a much greater extent than its stainability with the Feulgen nucleal stain. She points out that, "The Methylgreen stainability of chromatin on cytological preparations has been shown to be dependent upon the DNA being in a certain physical state, which for convenience may be referred to as "highly polymerized" (Kurnick, (22): Pollister and Leuchtenberger, (26)). By simple

experiments the nucleic acid can be changed so that it no longer stains with Methylgreen (i.e. it is depolymerized), although still capable of giving the full Feulgen reaction".

In the writer's experience the behavior of whole nuclei as well as of nuclear bodies range widely through many more physical shapes and physical states than we would expect. Chromatin (DNA) may take on a solid almost crystalline form, as seen in spear- or needle-like structures formed by spleen myeloblasts under influence of foreign serum (Engelbert (11, Fig. 15)). These spear-like structures remind one of Auer rods as seen in illustrations (Diggs *et al.* (6, Fig. 22 and 33)).

At other times the nucleus releases spherical bodies that appear to contain an almost fluid, homogeneous substance which stains densely in fixed preparations. Nuclear bodies containing a semifluid, homogeneous substance can be seen in avian blood, or in mammalian tissue (Engelbert (unpublished)). In avian blood, such spherical bodies may be found within a common cell boundary. Later the spheres may fuse and eventually rod-like bodies emerge from the apparently homogenous substance. Structures similar to the two stages described above are found in human blood cells and can be seen illustrated in Diggs *et al.* (6, Pl. XXII, D and Fig. 32, C). Diggs calls the dark bodies in the first illustration "pycnotic nuclei", and labels C in Fig. 32 "plasmocyte in mitosis". The appearance of the spherical semifluid bodies before they leave the nucleus may resemble Russell bodies (see Diggs *et al.* (6, Fig. 30)). Howell-Jolly bodies (Diggs (6, Pl. XXI, H and K)) nuclear fragments (Diggs (6, Pl. XXI, D)) and other nuclear bodies (Engelbert (11)) should be examined for possible similarities. There is obviously much of the nuclear life history that we do not know. Many nuclear phenomena, which in the light of our present knowledge appear as "nuclear breakdown", should be thoroughly investigated, to see what really happens during "the nuclear breakdown". The importance of DNA for protein synthesis and restoration of nuclei deprived of DNA was shown recently in isolated nuclei by Allfrey, Mirsky, and Osawa (1). These authors claim that, "The deoxyribonucleic acid of the nucleus plays a role in amino acid incorporation. Protein synthesis virtually ceases when the DNA is removed from the nucleus, and uptake resumes when the DNA is restored".

The viscosity of deoxyribonucleoprotein gels was investigated by Bernstein (2). He claims that "The high anomalous viscosity of the deoxyribonucleoprotein gels is not attributable solely to the presence of highly polymerized DNA. The gel structure is determined largely by intermolecular associational bonds in which proteins are primary participants". Such investigations of the physiology and chemistry of the nucleus and the nature of DNA help our understanding of the function and behavior of the living nucleus. We might with advantage look for behavior patterns in living nuclei that may seem abnormal and "degenerative" at present, but which might lead us to much new knowledge in cytology. The nuclear life history and the behavior and activity of the interphase nucleus holds the key to many cytological and histological problems.

Acknowledgments

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EVOLUTION IN THE FIELD CRICKET, ACHETA ASSIMILIS FAB.[1]

R. S. BIGELOW

Abstract

Progeny of northern spring field cricket adults lay non-diapause eggs, undergo nymphal diapause, and overwinter as nymphs. Progeny of northern fall adults lay diapause eggs, do not undergo nymphal diapause, and overwinter as eggs. The two populations cannot interbreed freely in the field owing to a temporal difference in breeding seasons; they did not interbreed in the laboratory. Rearing experiments show that the developmental differences are genetically based rather than environmentally conditioned, and it is, therefore, unlikely that hybrids would be viable even if they were produced in the field. Consequently these two populations behave as good species. Field crickets from Virginia developed much more rapidly than did spring crickets from Quebec. Quebec spring males and Virginia females produced hybrids with developmental rates intermediate between those of their parents. More female than male hybrids were produced, and the females developed more rapidly than did male hybrids. Offspring were produced by hybrid females and Quebec spring males, but not by hybrid females and Virginia males. Partial, but incomplete reproductive isolation exists between Quebec and Virginia field crickets. A possible mechanism of sympatric speciation in insects is discussed.

Introduction

The name *Acheta assimilis* Fabricius (1775) was first applied to a field cricket from Jamaica. Subsequently, many morphological differences between North American field crickets were noticed, and a number of "varieties" and "species" were given formal names of their own. Eventually, all this morphological evidence broke down; Rehn and Hebard (7) listed 45 synonyms under the original name. All this morphological study had failed to demonstrate the presence of more than one species among field crickets.*

Field observations, however, continued to suggest that field crickets might not be as homogeneous taxonomically as their summary inclusion under one specific name implied. Criddle (2) described two "races" in Manitoba which he called the spring cricket, *Gryllus assimilis pennsylvanicus* Burm., and the autumn cricket, *G. assimilis luctuosus* Serv. This conclusion was drawn primarily from the observation that one adult population is present in the field from about May 1 to August 1 and is then replaced by another, which appears about August 1 and remains until the onset of winter. Criddle stated that the spring cricket overwintered in the nymphal stage while the autumn cricket overwintered in the egg, but he did not discuss the evidence upon which this conclusion was based. Although he stated that there was no evidence of interbreeding between the two seasonal populations, he failed to demonstrate conclusively that the fall population was not merely a second generation derived from the spring population. To many, the absence of distinct structural differences could be taken as evidence of interbreeding.

[1]Manuscript received November 7, 1957.
Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Macdonald College P.O., Que., Canada. Journal Series No. 417.
*The term "field cricket", as used in this paper, excludes the genus *Nemobius*.

Severin (8, 9) also suggested that there might be two "biologic races" in South Dakota, one hibernating in the egg, the other in the nymphal state. Like Criddle, however, he did not eliminate the possibility of two generations rather than two "races".

In Louisiana, Folsom and Woke (3) observed that field cricket eggs laid in late April and early May produce adults in late July and early August, and that eggs from this second generation produce nymphs that overwinter. Folsom and Woke had actually observed one generation succeed the other in the insectary. This evidence did not support the suggestions of Criddle and Severin.

Cantrall (1) again suspected that, in Michigan, the spring and fall populations "might actually represent two different variants each having an annual cycle but mating at different times". He was aware of the observations of Folsom and Woke, however, and also considered the possibility "that the two populations were merely successive generations of a single variant which was two-brooded". Cantrall attempted to solve the problem, which he referred to as one of the most difficult of those he encountered in his work on the George Reserve, but he was concerned at the time with many other species as well, and his results were not conclusive.

Fulton (4) reported four "ecological races" in the southeastern United States, all apparently reproductively isolated from one another. His evidence included crossbreeding tests, examination of spermathecae for presence or absence of sperms, and ecological observations. Fulton reported that one of these "races" (the "triller") was bivoltine and that the other three ("woods", "mountain", and "beach" crickets) were univoltine.

North American field crickets, then, seem to include a complex of geographical races and possibly sibling species, but this complex is still very imperfectly understood. More definite establishment of the true status of the spring and fall populations in the north, and a direct comparison of northern and southern populations should be a step toward a fuller understanding of this complex.

Northern Spring and Fall Populations

Field Observations

Field observations in Quebec agree remarkably well with those made by Criddle, Severin, and Cantrall in Manitoba, South Dakota, and Michigan. In all these regions adult field crickets are present in the field during two more or less distinct periods. In all four regions the spring population dies off near the end of July and the fall population matures, rather suddenly, in early August. The presence of breeding field crickets can be detected readily in the field from the very audible chirping of the males. Near the end of July this chirping almost, but not quite, disappears altogether. In all four regions there is apparently no difference in habitat preference between the two populations. The fall adults succeed the spring adults in the same fields, and under the same objects. In Quebec, as in Manitoba, the spring

adults are less gregarious and much more difficult to capture than are the fall adults. These differences in behavior, and the suddenness of the appearance of the fall adults, suggest two discrete entities rather than two successive generations.

If the fall adults are the progeny of the spring adults, one would expect that the ratio of large nymphs to adults would change gradually over a period of 2 months, corresponding to the period during which spring adults have been ovipositing. This is not the case. About two weeks after the appearance of the first fall adults, large nymphs are extremely difficult to find. The suddenness of the appearance of the very numerous fall adults suggests derivation from overwintered eggs that have hatched more or less simultaneously in the spring.

The earliest chirping of spring adults that I heard in Quebec took place on June 10 in 1956 and on May 12 in 1957. The transition from spring to fall adults took place around August 1–10 in 1956, and around July 24–31 in 1957. If the fall adults were the progeny of the spring adults they must have matured in about 50 days in 1956, or in about 75 days in 1957; the eggs that produced them must have been laid during a single 2-week period, or else the incubation period of these eggs must have decreased as the season advanced. If matured progeny of spring adults mingled with fall adults in the fall, they must have matured in less than 112 days in 1956, or 141 days in 1957. The rate of development of the progeny of spring adults, then, bears directly on the problem.

Developmental Periods

On May 29 and 31, 1956, a total of 61 last stage nymphs were collected from beneath stones and other debris. At this time no mature adults had been heard chirping in the field. From one to five females were placed with one to three males in each of nine 1-gal. glass candy jars, and each culture was provided with a tray of food (balanced pig-food ration), a supply of water in a vial plugged with cotton wool, and strips of paper towel. Forty-five of these nymphs (75%) matured on June 9 and 10 within a period of 36 hours. The last of the remaining 15 matured on June 15 (i.e. the entire 60 matured within a 6-day period). Each culture was supplied with a tray of moist sand and checked daily for eggs. After a 5-day preoviposition period eggs were laid copiously in all cultures. This continued until the end of July, when individuals began to die off. Most of the original 60 were dead before August 10, and very few eggs were laid after that date (although one female survived until August 23). Eggs were removed, sand and all, either daily or once every 2 days, and incubated at 100% relative humidity in tightly closed screw-top jars under the following conditions: (1) in an outdoor insectary, (2) at room temperature, and (3) at constant temperatures of 73 ± 2° C., 82 ± 2° F., and 91 ± 2° F. A total of 133 egg samples were incubated, and a total of over 2000 nymphs emerged.

In 1956, these progeny of spring adults were reared in the insectary, and at room temperatures. In 1957, progeny of spring adults were reared in a 24 in. × 18 in. × 16 in. glass-walled cage with an attached water supply for automatic maintenance of moist sand. (Pig food was replaced by guinea-pig food in 1957.) Temperatures in this cage varied between 65° F. and 86° F., with a mean daily temperature of 75° F. Temperatures recorded in field microhabitats during June, July, and August, 1957, varied between 44° F. and 101° F., with a mean daily temperature of 74° F., i.e., mean daily temperatures in the field differed from those in the laboratory by only 1° F.

Developmental periods observed at temperatures comparable to those in field microhabitats are summarized in Table I.

TABLE I

DEVELOPMENTAL PERIODS OF PROGENY OF SPRING FIELD CRICKET ADULTS, IN DAYS

	Outdoor insectary 50–80° F., mean daily 68° F.	Laboratory 65–86° F., mean daily 75° F. or at 73 $\pm$ 2° F.	Required at temperatures (44–101° F., mean daily 74° F.)	
			If fall adults progeny of spring adults	If spring and fall adults interbreed in autumn
Preoviposition	5 or more	5		
Incubation of eggs	28–40	16–34		
Nymphal development	Well over 62	Over 122		
Total	Less than half grown in 95–109	Not past eighth instar in 143–161	50–75	122–141

Under these conditions, no nymphs had passed beyond the eighth instar* by mid-October either in 1956 or in 1957. From mid-September to mid-October, both years, mean laboratory temperatures were higher than those in the field, but no nymphs reached maturity during this period nevertheless. These results strongly suggest that the progeny of spring adults do not mature in the field before the onset of winter.

Comparison of First and Second Generation Incubation Periods

On November 20, 1956, about 15 adult females and eight males derived from spring adults (reared through the later instars at 82° F.) were placed in three 1-gal. glass candy jars. Eggs were collected daily, or every 2 or 3 days. These eggs were incubated at room temperatures, 82° F. and at 91° F. Oviposition continued in these cultures until the last female died on January

*The smaller, distended, grayish-appearing nymphs preparing to molt are readily distinguishable from the larger, compact, brown or jet-black newly molted nymphs of the succeeding instar. Instars were thus segregated daily or every 2 days and specimens of each were preserved in alcohol. Determination of instars in other rearing experiments was then done either directly, or by comparison with these preserved specimens.

TABLE II

INCUBATION PERIODS OF EGGS LAID BY SPRING ADULTS (FIRST GENERATION), AND BY THEIR PROGENY (SECOND GENERATION)

	Room temperature	82 ± 2° F.	91 ± 2° F.
First generation	16–29 days, majority 20 days	12–23 days, majority 14 days	9–16 days, majority 10.5 days
Second generation	18–28 days, majority 22 days	11–17 days, majority 14 days	9–15 days, majority 11 days

30. In Table II the average incubation periods of these second generation eggs are compared with those laid by spring adults. Incubation periods were practically identical in both generations. No indication of a tendency to lay diapause eggs was observed in the second generation.

Many hundreds of eggs were laid in the laboratory by fall crickets that had been collected as large nymphs late in July. Samples of these eggs were incubated at different temperature conditions. At room temperature, nymphs appeared in four samples after incubation periods ranging from 62 to 87 days. (Eggs from spring adults hatched in about 20 days under these conditions.) Many samples of fall eggs were refrigerated at about 40° C., and some were removed to 82° F. at intervals during the winter of 1956–57. Nymphs hatched from 15 of these samples, after an incubation period averaging about two weeks from the date of removal from refrigeration.

A second generation of about 15 fall adults oviposited in the laboratory from February 4 to March 15, 1957. These eggs behaved like those of the preceding generation; none hatched in less than 2 months at room temperatures.

No indication of a change in the incubation period of the eggs was observed in the second generation of either spring or fall progeny.

Retardation of Nymphal Development

When the progeny of spring adults reached the seventh instar they almost invariably passed into a state of retarded development. At room temperatures, 10 to 35 days were spent in the seventh instar. Individual variation was extensive, but the majority of nymphs spent about 18 to 25 days in this instar, and very few passed through it in less than 18 days. The duration of each of the preceding instars was regularly about six to nine days, or about seven days on the average. At a constant 82° F. about five to seven days were spent in each of the first six instars and seven to 43 days, or an average of 20 days, were spent in the seventh. The same phenomenon took place in the second generation derived from spring adults. The duration of the eighth instar was similar to that of the seventh and nymphs that passed through the seventh instar relatively quickly remained for long periods in the eighth.

TABLE III

COMPARISON OF DEVELOPMENTAL PERIODS, FROM HATCHING TO MATURITY, OF SECOND GENERATION SPRING AND FALL NYMPHS (ABOUT 25 NYMPHS FROM EACH POPULATION WERE INVOLVED)

	$82 \pm 2°$ F.	$91 \pm 2°$ F.
Spring	76–100 days	50–60 days
Fall	45–52 days	28–42 days

Retardation of development in the later instars did not take place in the progeny of fall adults. Duration of nymphal development in spring and fall progeny is compared in Table III.

Growth retardation in the progeny of spring adults was not conditioned by the onset of lower temperatures or by changes in light:dark ratio in September. It was observed in the spring progeny at various temperature conditions and in midwinter in the laboratory; it was never observed in fall progeny.

Crossing Experiment

During the winter of 1956–57 a second generation of adults, derived from both the spring and fall populations, were crossed in the laboratory. About 15 females and 5 males from each population were involved in this experiment. Eggs were collected from January 2 to February 28, 1957. Sperms were present in the spermatheca of a spring female on February 27, and eggs were laid (rather sparingly) in all cultures. None of these eggs hatched. Samples were incubated at room temperatures, 82° F., 91° F., and at 40° F. for varying periods. Subsequent careful examination of the eggs in all cultures revealed no indication of a developing embryo. During the same period, eggs hatched in both pure spring and pure fall cultures.

Comparison of Northern and Southern Populations

Between March 29 and April 1, 1957, several hundred immature field crickets were collected in Virginia and North Carolina, along a route that passed through Remington, Apple Grove, and Blackstone in Virginia; Henderson, Wake Forest, Raleigh, and Garysburg in North Carolina; and Courtland, Wakefield, Jamestown, Saluda, and Tappahannock in Virginia on the return trip. At this time maples, willows, and elms were beginning to show leaves and no crickets were heard chirping in the field. Several adults were taken near Blackstone, Virginia, and a few small nymphs (fourth or fifth instar) were taken at scattered localities. The majority of the nymphs collected were between the sixth and the ninth instars.

The southern males and females were segregated and kept at about 60° F. in the laboratory until field-collected nymphs from Quebec had begun to mature in May. Development of the southern nymphs was very slow at 60° F., but they appeared not to be adversely affected otherwise.

About two dozen nymphs were placed in each of four glass-walled cages with an attached water supply for automatic maintenance of moist sand, as follows:

Cage VV: Virginia females × Virginia males.

Cage VQ: Virginia females × Quebec spring males.

Cage QV: Quebec spring females × Virginia males.

Cage QQ: Quebec spring females × Quebec spring males.

(Virginia males and a few third generation laboratory-reared Quebec fall males, and Virginia females and one third generation laboratory-reared Quebec fall males, were placed in two other cages. No nymphs have appeared in these cages.)

Environmental conditions were practically identical in all these cages throughout the summer. First instar nymphs began to appear in cage VV on about June 7, and in cages VQ and QQ on about June 19. In cage QV, Quebec spring females carrying spermatophores were observed, but eggs were laid sparingly, and no indications of developing embryos were observed in any of these eggs, after careful examination of all that could be found.

Large numbers of nymphs were produced in cages VV, VQ, and QQ and striking differences in their rates of development became obvious as the season advanced. The pure Virginia nymphs developed much more rapidly than did the hybrids or the pure Quebec spring nymphs. Adults began to appear in cage VV on August 1; by October 1 these had oviposited and died off. A few small second generation nymphs were present in this cage on October 15. Adult females appeared in cage VQ on September 16, at which time the stages of development in this cage varied from these adults to very small third or fourth instar nymphs. The majority of the hybrids were at intermediate nymphal stages. In cage QQ all nymphs were uniformly small in mid-October; none had passed beyond the eighth instar. Throughout the season the hybrids were conspicuously intermediate in size between the large pure Virginia and the small pure Quebec spring nymphs. Large males were conspicuously rare among the hybrids. Of 27 nymphs reared to maturity at 82° F., 25 were females and only two were males. Furthermore, these two males matured over 10 days after the last female had matured. On October 15 the hybrids remaining in rearing cage VQ consisted of 10 adult females, nine large female nymphs (eighth or ninth instar) and 11 small nymphs in the sixth or seventh instar, all of which were males. Not only were the females much more numerous, but their development was much more rapid.

Several of the female hybrids reared through to maturity at 82° F. were backcrossed to the parental strains. Offspring were produced by hybrid females and Quebec spring males, but not by hybrid females and Virginia males.

At mean daily room temperatures of about 75° F., then, the pure Virginia nymphs began to mature 54 days after hatching; the most advanced hybrids began to mature 89 days after hatching; and the pure Quebec spring nymphs were still in the eighth instar 118 days after hatching.

Despite their more rapid development, the Virginia crickets passed through 11 nymphal instars while the Quebec spring crickets passed through only nine.

Detailed morphological comparisons have not yet been made, but differences exist between all stages (e.g. the Virginia crickets tend to be larger, the nymphs more gray and less shiny black than the Quebec spring crickets).

In the Virginia males the song was a subdued and rather extended "trill" that contrasted sharply with the loud, intermittent chirps of the Quebec crickets. This "trilling" was the only song heard in any of the cages containing southern crickets.

Discussion

Northern Spring and Fall Populations

Daily mean temperatures in field microhabitats were very similar to those in laboratory cages, in which none of the progeny of spring adults matured before the onset of cold weather in the field. The main difference between field and laboratory temperature conditions was a greater fluctuation in the field. Under conditions of fluctuating temperature in the outdoor insectary, development was not accelerated but was, if anything, decelerated. At constant temperatures that were approximately equal to mean daily room temperatures, developmental periods were similar. It is reasonable to conclude, therefore, that development at room temperatures in the laboratory was as rapid as that in the field. If so, the spring and fall populations do not interbreed (or do so to a very slight extent), and are not two successive generations of a bivoltine species.

If the spring and the fall populations belong to a single bivoltine species, either the successive generations alternately produce diapause and nondiapause eggs and nymphs, or else these developmental phenomena are conditioned by seasonal changes in temperature, light:dark ratio, etc. Neither of these phenomena was observed in the laboratory. Spring adults never laid diapause eggs and always produced diapause nymphs. Fall adults always laid diapause eggs and never produced diapause nymphs. These developmental characteristics are genetically ingrained into each population and are not environmentally conditioned. They reveal a fundamental genetical difference between the two populations which, though useless as an aid in distinguishing between dead adult specimens, is as good evidence of specific distinction as any single structural difference.

If interbreeding does not take place in the field, the fact that it *can* take place under artificial conditions cannot prove conspecificity. Whether or not the spring and fall populations *can* interbreed, the essential fact remains that they do not do so in nature. The temporal difference in their breeding periods isolates them as effectively as would a spatial separation of many miles. It is difficult, even in the laboratory, to obtain adults of the two populations in breeding condition at the same time. This difficulty is more significant evidence of reproductive isolation in nature than is the ability to interbreed under artificial laboratory conditions.

If hybrid progeny can be produced, and if winters in northern North America were to become mild enough to allow all stages to overwinter, it is at least conceivable that the present developmental and breeding season differences might break down. However, the production of fertile hybrids has not yet been demonstrated conclusively, and winters in northern North America will probably continue to be severe for long enough to allow a genetical consolidation of even greater differences between the two populations. If interbreeding occurs between northern and southern races in such a way as to allow an exchange of genes between the northern spring and fall populations through a roundabout "ring" of southern races, it might not be unreasonable to consider the two populations as conspecific. The extent of gene exchange through such a "racial ring" must be very slight, however, and until such a "ring" has been demonstrated, it is advisable to assume that it does not exist.

A considerable body of evidence, then, suggests that the northern spring and fall populations differ genetically and do not interbreed freely at the present time. The distinctive differences between the two populations are more likely to become further consolidated than they are to break down through any future gene exchange. Therefore, these two populations should be regarded as two distinct species, however similar they might be morphologically. If species are to be defined in terms of reproductive isolation, these two populations should not be called "races" or "variants", but should be clearly recognized as two distinct species. Criddle (2) was correct when he suspected "this is an instance when similarity of structure must not be taken too literally."

Northern vs. Southern Populations

Although the only song heard in any of the cages containing southern crickets was the very distinctive "trilling" that identified these crickets as Fulton's "trillers", it is of course likely that southern "woods" crickets were also present. The appearance, in mid-October, of a pure Virginia male (reared at 82° F.) that chirped like Quebec crickets makes this conclusion almost certain. It is also possible that some may have been "mountain" crickets but this is less likely since the range of "mountain" crickets is more toward the western parts of Virginia and North Carolina, rather than the eastern parts of these states where the collecting was carried out. The very rapid development of all the pure southern progeny, however, suggests that these were all progeny of "trillers" (the only southern form reported by Fulton as having two generations annually). The very striking intermediacy in developmental rates of the hybrid progeny between those of the pure southern and pure northern progenies strongly suggests that the interbreeding took place between "triller" females and Quebec spring males. If these hybrids had been derived from Quebec spring males and "woods" females, one would expect their developmental rates to have been more like those of both parental forms, neither of which produce nymphs that mature in the field before the following spring.

The presence of immature hybrid males and females in cage VQ on November 1, 1957, however, suggests that these might have been produced by Virginia "woods" females and Quebec males. If so, both southern strains crossed with Quebec spring males, one cross producing females only, the other more or less equal numbers of males and females with developmental rates similar to those of their parents. This would suggest that fewer genetical isolating factors exist between "woods" crickets and Quebec spring crickets than between the latter and "trillers".

It would be most curious if all the hybrid females proved to be derived from "trillers" and Quebec males, while the slowly developing hybrid males were derived from "woods" females and Quebec males. If this were the case, both crosses would have produced progeny of one sex only. According to Haldane's rule: "when in the F_1 offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterozygous sex" (Haldane (5)). In crickets, males are the heterozygous (i.e. heterogametic) sex. Therefore, rare or ill-adapted males, *but not males only*, are to be expected from interracial crosses of crickets. It is, therefore, reasonable to conclude that crossing took place between "trillers" and Quebec spring males, and also that genetical factors tending toward the production of reproductive isolation have begun to appear between these two forms. Since copulation took place between Quebec spring females and Virginia males, their failure to produce offspring may have been due to the presence of intersterility between them. Virginia males and hybrid females also appeared to be intersterile.

Fulton's tests showed no tendency for "trillers" to interbreed with "woods" crickets. Assuming that interbreeding took place between "trillers" and Quebec spring males, as the evidence strongly suggests, it is interesting to observe that reproductive isolation seems to be more complete between the sympatric southern forms than it is between at least one of these and a northern form. Although differences in song, size, color, and rate of development indicate complex differences in their genetical constitutions, the northern and southern forms produced fertile hybrids. This is in accord with the theory that reproductive isolation is more likely to be present between closely related sympatric species than between closely related allopatric species.

The production of fertile hybrids suggests that the Virginia "trillers" and Quebec spring crickets are conspecific. On the other hand, the apparent infertility between southern males and both hybrid and northern females, and the unequal sex ratio of the hybrids, suggest that a free interchange of genes could not take place between the two forms. It is very likely that the hybrids would be less well adapted in the field than either parent population. The intermediate and variable developmental periods of these hybrids are not likely to be advantageous in either the north or the south. If this can be assumed, it is unlikely that the peculiarities of the two populations would be swamped, even if they lived sympatrically. If the two forms are thus destined to evolve independently they should be regarded as two distinct

species. It is clear, in any case, that these two forms represent an intermediate stage of speciation, and this fact is more significant from an evolutionary point of view than is the question as to whether speciation has, or has not, been completed.

On the Possibility of Sympatric Speciation

McGregor (6) reported all stages of field crickets overwintering in the mild conditions of the Imperial Valley of California. Fulton's "mountain" cricket of the southeastern United States apparently overwinters primarily in the nymphal stage, but also in the egg to some extent; his "beach" cricket apparently overwinters primarily in the egg, but also to some extent in the nymphal stage. In Manitoba, Michigan, South Dakota, and Quebec the spring population overwinters exclusively in the nymphal stage and the fall population exclusively in the egg.

Where winters are mild, all stages overwinter. Where winters are less mild, but not severe, there is a tendency to overwinter *either* in the nymphal *or* the egg stage, but still to overwinter in both stages. Where winters are severe, overwintering is accomplished either exclusively in the egg or exclusively in the nymphal stage.

Reproductive isolation is usually assumed to have been preceded by geographical isolation. This is certainly the most logical hypothesis to account for the origin of most existing species, particularly among vertebrates. The impressive numbers of invertebrate species, however, particularly insects, suggest that speciation in these groups might be taking place both with and without the aid of geographical isolation. It is, therefore, interesting to speculate on the possible origin of reproductive isolation between spring and fall crickets of the north in the light of the evidence outlined above.

If the mild winters in a given region, where crickets are overwintering in all stages (e.g. Imperial Valley of California) were to gradually become more severe; or if conspecific cricket populations gradually migrated from such a region toward a colder region, winter mortality would tend to increase. If some stages were less able to survive winter conditions than others, selection would favor those individuals which produced offspring that reached a winter-resistant stage late in the fall. If intermediate stages were less winter-resistant than either the egg or late nymphal stages, selection would tend to follow two courses: (1) toward the production of winter-resistant large nymphs, and (2) toward the production of winter-resistant eggs.

Overwintered eggs hatch, and overwintered nymphs mature, more or less simultaneously in the spring. Two *temporally isolated* populations are thus produced. The overwintered nymphs mature, mate, and oviposit while the nymphs that hatch from the overwintered eggs are developing toward maturity. If the first population of adults dies off before the second population of adults matures, reproductive isolation between the two populations could not be more complete. In time, the genetical differences involving the

production of diapause eggs but not diapause nymphs on the one hand, and diapause nymphs but not diapause eggs on the other, will tend to increase between the two populations. More genes will tend to become involved in producing and perfecting diapause in either one stage or the other. Eventually hybridization, even if it were possible, would produce hybrids tending toward diapause in both the egg *and* the nymphal stage, toward a "semi-diapause" in one or both of these stages, or toward an absence of diapause in either stage. None of these eventualities is likely to be advantageous in a region of severe winters. The hybrids will either be out of phase at the onset of winter, or will be unable to survive winter conditions in any case. Speciation might thus be accomplished sympatrically from beginning to end, without the aid of any geographical isolation whatsoever.

Such a phenomenon seems to exist in all its essential stages within the North American field cricket complex. Hybridization between geographically separated field cricket populations is still possible, but tends to produce hybrids whose developmental rates are out of phase with seasonal conditions in either parental region. Hybridization between seasonally isolated populations in the north is either impossible, or it occurs very rarely. If it occurs, it is unlikely to produce hybrids capable of overwintering in the field.

If speciation can be thus accomplished sympatrically by field crickets, it is not unreasonable to assume that it might have been accomplished by other insects as well (e.g. in *Arphia conspersa* and *pseudonietana* among grasshoppers, or in the spring and fall cankerworms among moths).

Conclusions

The field cricket complex includes two good species in the north, and possibly four good species in the southeast. Reproductive isolation is probably present, though incomplete, between southern "trillers" and northern spring crickets. Similar intermediate stages of speciation probably exist between other geographically separated populations. Further study of the field cricket complex should throw light on mechanisms of insect speciation generally as well as on the microtaxonomy of field crickets in particular. The taxonomic history of the field cricket complex is another example suggesting that morphological study should follow rather than precede biological study in attempts to solve microtaxonomic problems.

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PARASITES OF SOUTH PACIFIC FISHES

I. INTRODUCTION, AND HAEMATOZOA[1]

Marshall Laird[2]

Abstract

During 1952–54, fishes were collected and examined for parasites (particularly those of the blood and gills) at various localities ranging from Sydney, Australia, through the island groups of Melanesia to Tarawa, Gilbert and Ellice Islands Colony. Most of the specimens were obtained by poisoning intertidal zone pools on coral reefs or rocky coasts, the remainder being from fresh-water or brackish sources. A total of 1121 fishes of at least 122 species, 63 genera, and 27 families were dealt with. This paper, the first of a series detailing the findings, includes a complete list of the material, an account of the techniques employed, and new host and locality records for *Haemogregarina bigemina* Laveran and Mesnil and *H. mugili* Carini.

Although there is a voluminous literature on the parasitology of fishes of the North Pacific, from the coasts of Asia and Japan to the Galapagos and the Americas, and much is now known of this subject from temperate Australasian waters, remarkably little has been published concerning tropical South Pacific fish parasites. A single haemogregarine-infected blenny was found out of a total of 93 coral reef fishes of 11 species examined in Fiji on an earlier occasion (6), suggesting a much lower incidence of haematozoa than prevails in intertidal zone fishes of temperate New Zealand waters, where two species of haemogregarine and a trypanosome were recorded from 69 fishes of six species out of 458 examples of 10 species dealt with (8). As regards other parasites, a myxosporidian has been described from a Fijian shore fish (5), and four monogenetic (10) and two digenetic (11) trematodes are known from Fiji. These few records constitute the available data on the subject, from a vast area notable for the richness and diversity of its fish fauna.

The opportunity was thus taken to examine fish for parasites wherever possible, during 2 years spent on malariological studies in the tropical South Pacific. Also, immediately before the start of this project, 2 weeks were spent at Norfolk Island for the specific purpose of investigating the parasitology of fishes of the intertidal zone; and soon after the conclusion of the tropical field work in 1954, a visit to Sydney provided an opportunity for a further intertidal zone collection which yielded more data for purposes of discussion and comparison additional to those already to hand from temperate waters in New Zealand (8).

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Contribution from the Institute of Parasitology, McGill University, Macdonald College, Macdonald College P.O., Que., Canada. The visit to Norfolk Island was made possible by a Hutton Grant from the Royal Society of New Zealand, all other field work being undertaken during a research project initiated by the Royal New Zealand Air Force and supported by a grant from the N.Z. Department of Scientific and Industrial Research.
[2]Institute of Parasitology, McGill University.

Methods and Material

Field trips were made to various parts of the Pacific from a base at Suva, Fiji, during 1952–54. Some of these trips were of very short duration, and preoccupation with malariological work left very little time for side projects such as the present one. It was seldom practicable to arrange for collections from pelagic fishes, and furthermore, on-the-spot identification of the hosts being out of the question, this would have involved considerable difficulties in the preservation and storage of relatively large specimens, for all long-distance transportation was by air. On the other hand, as the malariological studies had to be undertaken in coastal areas or from a coastal base, it was always possible to use rotenone (5%) for immobilizing fish in the lower reaches of streams and in intertidal pools. This procedure both yielded material from a biotope already intensively investigated in New Zealand from which considerable data for comparative purposes were thus already to hand and resulted in the collection of fish specimens of such small size that they presented no preservation or storage problems.

Pelagic fishes, other than those occurring as juveniles in the intertidal zone, were thus neglected altogether. Most of the specimens were obtained at low tide from pools on coral reefs or rocky coasts, a small minority being from brackish or fresh-water habitats.

Thin blood films were made from the heart, care being taken to wipe away all pericardial fluid before the incision was made. Absolute methyl alcohol was employed for fixation, and Giemsa for staining. Gill smears were made on $\frac{7}{8}$ in. square cover slips (No. 1). There was usually sufficient mucus present to ensure that parasites adhered to the glass. Where there was not, and whenever smears were made from the intestine, gall bladder, or urinary bladder, cover slips bearing a thin film of Mayer's albumen were used. All such smears were fixed in hot Worcester's formol – mercuric – acetic mixture as modified by Davis (3)—75 cc. saturated solution mercuric chloride, 20 cc. formalin (formaldehyde 40%), and 5 cc. glacial acetic acid. They were stained with Heidenhain's haematoxylin prepared by Shortt's rapid method (14)—95 cc. distilled water slowly boiled with 1 g. pure haematoxylin crystals, 5 cc. pure carbolic acid being added as the boiling point is reached. This stain is ready for use on cooling. It proved highly satisfactory, and retained its qualities for as long as 9 months at room temperature at Suva. Destaining was accomplished in a saturated aqueous solution of picric acid, and Stafford Allans' "Sira" mountant, hardening more firmly than Canada balsam and not becoming discolored under tropical conditions, proved a thoroughly satisfactory mounting medium.

Blood and gill smears were made from all the Norfolk Island fishes within a few minutes of death, and wherever possible internal organs, particularly the gall bladder and urinary bladder, were ligatured, dissected out, and preserved in formalin. There was no time for the special preparation of internal organs on subsequent field trips, and from an early stage attention was confined to the blood and gills unless circumstances allowed more detailed examination.

It soon proved that a high percentage of the fishes were without gill parasites; thus to avoid wastage of time in preparing and examining large numbers of negative cover slip smears, the practice was adopted of searching fresh material from the gills as soon as the catch was brought to the beach. The lamellae were scraped with the back of a scalpel, a drop of the fluid so obtained being covered and examined under a 10/0.25 phase objective and $\times$ 12.5 oculars. The resulting degree of magnification was sufficient for the detection of even the smallest Protozoa infesting the gills. For field use, the integral illuminator of the microscope, a Zeiss-Winkel GF 525, was powered from a 6 v. car battery. Permanent preparations were made only from gills thus found to be parasitized, a register of negatives of course being maintained. A fresh drop of blood obtained and covered at the same time as the gill scraping was searched for trypanosomes, which are often present in the circulation in such small numbers that they are liable to be overlooked in surveys based upon thin films alone. However, it is extremely difficult to detect light infections with haemogregarines by fresh blood examination, even with the aid of phase contrast. A thin film was thus made from each fish dealt with, stained with Giemsa, and examined for half an hour before being discarded as negative. A great number of slides being involved, this task was put aside and resumed from time to time as opportunity afforded and was not completed until towards the end of 1956.

In all cases, a small aluminum tag bearing a die-punched number was tied to representative specimens of each species of fish examined, these specimens being preserved in formalin for subsequent identification and the number being applied to all relevant parasitological material.

The field trips are listed hereunder in chronological order. Fig. 1 shows the location of the collecting stations, the letters assigned to which are employed in the list of fishes in the following section.

1. Norfolk Island, February 3–17, 1952.
   (A) Coral reef off Kingston, Sydney Bay.
   (B) Sandstone pools, Kingston foreshore.
   (C) Rock pools, Cascade Bay.
2. *Viti Levu*, Fiji, June 15, 1952.
   (D) Pond alongside Rewa River, Nausori.
3. *Aneityum*, New Hebrides, August 5, 1952.
   (E) Fringing reef, Anelgauhat.
4. *Venui*, off S. Espiritu Santo, New Hebrides, September 6, 1952.
   (F) Intertidal pools in coral rock formation.
5. *Viti Levu*, Fiji, September 21, 1952.
   (G) Hoofprint pools bordering marsh, Singatoka.
6. New Caledonia, December 4, 1952.
   (H) Ponded stream, Koumac.
7. New Caledonia, December 6, 1952.
   (I) Brackish pond, Voh.

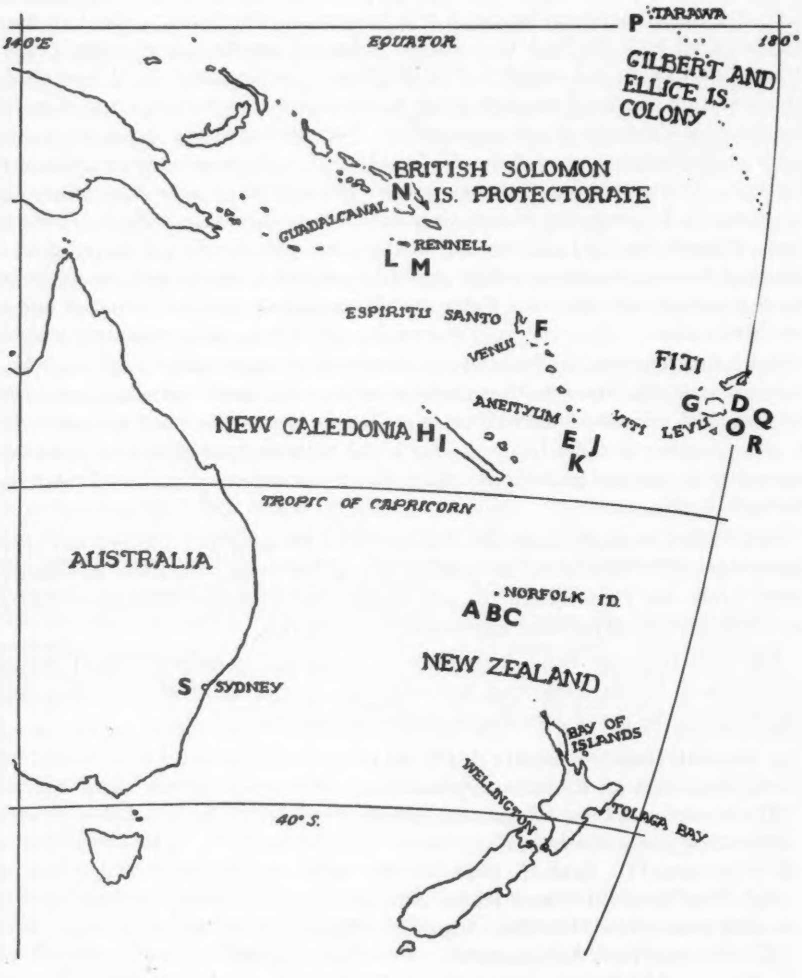

FIG. 1. Sketch map indicating location of collecting stations.

8. *Aneityum*, New Hebrides, March 2–7, 1953.
 (J) Fringing reef, Anelgauhat.
9. *Aneityum*, New Hebrides, March 5, 1953.
 (K) Tidal stream, Anelgauhat.
10. *Rennell*, British Solomon Islands Protectorate, August 19, 1953.
 (L) Edge of (brackish) Lake Te-nggano, Niupani.
11. *Rennell*, British Solomon Islands Protectorate, August 20, 1953.
 (M) Fringing reef, Te-Uhungango.
12. *Guadalcanal*, British Solomon Islands Protectorate, September 11, 1953.
 (N) Stream-bed ponds, mouth of Poha River.
13. *Viti Levu*, Fiji, December 15, 1953.
 (O) Oxbow pond, bed of Nambukavesi Creek, Queen's Road, approximately 15 miles from Suva.
14. *Tarawa*, Gilbert and Ellice Islands Colony, January 28, 1954.
 (P) Shore reef, Bairiki.
15. *Makuluva*, off Lauthala Bay, Viti Levu, Fiji, January 2 and February 21, 1954.
 (Q) Barrier reef.
16. *Viti Levu*, Fiji, March 21, 1954.
 (R) Under coral blocks on mud flats, Suva Point, Suva.
17. New South Wales, Australia, June 24, 1954.
 (S) Sandstone pools, North Bondi, Sydney.

Species of Fish Examined for Parasites

All specific determinations were made by Henry W. Fowler, Curator of Fishes at the Academy of Natural Sciences of Philadelphia. The major classification follows the usage of the *Zoological Record*. The specific names are followed by the total number of examples collected, then by the relevant locality key letters, and the number from each in parentheses.

APODES

Muraenidae

Echidna polyzona (Richardson) 1: J (1)
Gymnothorax flavimarginatus (Rüppell) 1: A (1)
 meleagris (Shaw) 4: R (4)
 meleagris ercodes Jenkins 1: J (1)
 nubilis Richardson 3: A (3)
 pictus (Ahl) 4: E (1), F (3)
 undulatus Lacépède 3: J (2), Q (1)
 sp. 1: F (1)

Ratabouridae

Rataboura javanica (Kaup) 2: J (2)

Congridae

Forskalichthys cinereus (Rüppell) 5: J (5)

MICROCYPRINI

Cyprinodontidae (Poeciliidae)

Lebistes reticulatus (Peters) Introd. 4: H (3), K (1)

SOLENICHTHYES

Syngnathidae

Choeroichthys brachysoma (Bleeker) 1: Q (1)
Corythoichthys flavofasciatus (Rüppell) 1: E (1)

PERCOMORPHI

PERCOIDEA

Serranidae

Acanthistius serratus (Cuvier and Valenciennes) 3: S (3)
Cephalopholis argus Bloch, Schneider 2: P (1), Q (1)
Epinephelus melanostigma Schultz 2: M (1), P (1)
merra Bloch 36: E (4), J (7), Q (25)
Grammistes sexlineatus (Thunberg) 2: F (1), J (1)
Serranus daemelii Günther 1: B (1)

Duleidae

Dules munda (De Vis) 17: K (17)
taeniurus Cuvier and Valenciennes 3: F (2), S (1)

Pseudochromidae

Pseudochromis tapeinosoma Bleeker 15: Q (15)

Plesiopidae

Plesiops melas Bleeker 3: E (1), J (2)
nigricans (Rüppell) 5: E (1), J (3), Q (1)

Apogonidae

Apogon bandanensis Bleeker 1: J (1)
novemfasciatus Cuvier and Valenciennes 5: E (1), J (1), Q (1), R (2)
Apogonichthys auritus variegatus (Valenciennes) 3: J (3)
ocellatus (M. Weber) 1: E (1)
pendix Bleeker 4: J (4)
Rhabdamia cypselura M. Weber 1: J (1)

Centropomidae

Ambassis miops Günther 5: N (5)

Pomadasyidae

Pomadasys operculare (Playfair) 1: K (1)

Mullidae

Pseudupeneus taeniatus (Kner) 1: J (1)

Girellidae

Girella cyanea Macleay 5: B (5)
tricuspidata (Quoy and Gaimard) 2: S (2)

Chaetodontidae

Chaetodon auriga Forskål 1: Q (1)
lunula (Lacépède) 1: P (1)
miliaris (Quoy and Gaimard) 1: E (1)
Linophora vagabunda (L.) 2: J (2)

Pomacentridae

Abudefduf coelestinus (Cuvier and Valenciennes) 2: J (2)
polyacanthus (Ogilby) 1: B (1)
sordidus (Forskål) 24: A (10), B (3), F (10), M (1)
Chrysiptera amabilis (De Vis) 2: M (2)
assimilis (Günther) 4: J (4)
biocellata (Quoy and Gaimard) 9: F (1), J (5), M (2), Q (1)
elizabethae Fowler 13: Q (13)
glauca (Cuvier and Valenciennes) 96: F (1), J (25), M (3), P (16), Q (51)
leucopoma (Cuvier) 18: F (1), J (3), Q (14)
leucozona (Bleeker) 31: Q (30), R (1)
sapphira (Jordan and Richardson) 1: F (1)
uniocellata (Quoy and Gaimard) 1: E (1)
yamashinai (Okuda and Ikeda) 1: Q (1)
zonata (Cuvier) 1: Q (1)
spp. 3: F (1), J (2)
Dascyllus aruanus (L.) 1: J (1)
Parma polylepis Günther 6: B (6)
Pomacentrus fasciolatus Ogilby 1: A (1)
nigricans (Lacépède) 43: E (6), J (2), P (9), Q (26)
tripunctatus Cuvier and Valenciennes 2: J (2)
spp. 4: E (4)

Labridae

Halichoeres binotopsis (Bleeker) 6: A (2), F (4)
leparensis (Bleeker) 1: J (1)
margaritaceus (Cuvier and Valenciennes) 45: J (3), Q (42)
trimaculatus (Quoy and Gaimard) 17: E (2), F (1), J (14)
spp. 6: E (4), F (1), J (1)
Stethojulis albovittata (Bonnaterre) 2: E (1), F (1)
axillaris (Quoy and Gaimard) 8: J (1), Q (6), R (1)
strigiventer (Bennett) 1: E (1)
Thalassoma hardwicke (Bennett) 20: Q (20)
lutescens (Lay and Bennett) 1: Q (1)
umbrostygma (Rüppell) 5: A (1), M (4)

Scaridae

Scarus erythrodon Cuvier and Valenciennes 1: E (1)
sp. 1: E (1)

Acanthuroidea

Acanthuridae

Acanthurus triostegus (L.) 65: F (1), J (27), K (1), M (8), Q (28)

Gobioidea

Eleotridae

Belobranchus sp. 1: N (1)
Eleotris fusca (Bloch, Schneider) 13: M (5), N (5), O (3)
Eviota viridis (Waite) 96: A (40), C (55), Q (1)
spp. 7: F (5), J (2)
Hypseleotris cyprinoides (Cuvier and Valenciennes) 12: 0 (12)
Lairdina hopletupus Fowler 2: G (2)
Acentrogobius cauerensis (Bleeker) 1: R (1)
puntang (Bleeker) 1: R (1)
Awaous ocellaris (Broussonet) 7: N (6), 0 (1)
Bathygobius fuscus (Rüppell) 32: B (22), F (6), J (2), R (2)
Ctenogobius nebulosus (Forskål) 9: D (3), N (6)
sp. 1: 0 (1)
Gobius ornatus Rüppell 4: J (3), R (1)
spp. 4: K (3), N (1)
Itbaya nuda Herre 9: F (9)
Stenogobius genivittatus (Cuvier and Valenciennes) 4: N (4)
Stigmatogobius duospilus Fowler 1: I (1)
Zonogobius semidoliatus (Cuvier and Valenciennes) 3: F (1), J (2)

Blennioidea

Blenniidae

Atrosalarias fuscus (Rüppell) 5: E (4), F (1)
Cirripectus variolosus (Cuvier and Valenciennes) 20: A (20)
Lepidoblennius hoplodactylus Steindachner 9: S (9)
Petroskirtes kallosoma Bleeker 2: R (2)
elongatus Peters 2: J (2)
Rhabdoblennius snowi (Fowler) 51: A (39), B (12)
Salarias amboinensis Bleeker 2: F (2)
bellus Günther 1: F (1)
bilineatus Peters 9: F (9)
edentulus (Bloch, Schneider) 7: F (3), M (1), P (2), Q (1)
fasciatus (Bloch) 50: E (1), J (4), Q (45)

lineatus Cuvier and Valenciennes 2: B (1), F (1)
marmoratus (Bennett) 5: F (5)
periophthalmus Cuvier and Valenciennes 28: F (2), J (6), M (3), Q (17)
sinuosus Snyder 2: Q (2)
spp. 34: F (9), J (2), M (23)

Clinidae

Clinus perspicillatus Valenciennes 8: A (8)
Norfolkia lairdi Fowler 1: A (1)
Tripterygion annulatum Ramsay and Ogilby 1: S (1)
hemimelas (Kner and Steindachner) 1: Q (1)
punctulatus (Herre) 3: J (2), M (1)
rufopileum Waite 46: A (43), B (3)
spp. 6: E (4), J (2)

MUGILOIDEA

Mugilidae

Mugil oligolepis Bleeker 12: K (12)
sp. 1: N (1)

SCLEROPAREI

SCORPAENOIDEA

Scorpaenidae

Scorpaena lineagula Fowler 1: R (1)
sp. 1: E (1)
Scorpaenodes guamensis (Quoy and Gaimard) 2: Q (2)

PLECTOGNATHI

BALISTOIDEA

Balistidae

Rhinecanthus rectangulus (Schneider) 1: J (1)

PEDICULATI

ANTENNARIOIDEA

Antennariidae

Antennarius bigibbus Lacépède 3: J (3)
sp. 1: M (1)

In all, therefore, a total of 1121 fishes of at least 122 species, 63 genera, and 27 families were dealt with during these studies.

Haematozoa

One of the eight examples of *Clinus perspicillatus* and two of *Tripterygion rufopileum* from the coral reef off Kingston, Norfolk Island, were infected with *Haemogregarina bigemina* Laveran and Mesnil; and three of the six examples of *Awaous ocellaris*, one of the four of *Stenogobius genivittatus*, and the single *Mugil* sp. from Guadalcanal, were parasitized by *H. mugili* Carini.

Haemogregarina bigemina Laveran and Mesnil, 1901 (Fig. 2, *a*)

Both infections were light ones, less than one red cell per 10,000 being parasitized. Several schizonts were noted, those within lymphocytes producing up to four merozoites. The endoleucocytic schizogony of *H. bigemina* was elucidated from New Zealand material (8), and has since been confirmed by Saunders (13) from Florida, U.S.A. The characteristic, paired gametocytes (Fig. 2*a*) measure 9.8 to 12.4 μ by 1.4 to 2.0 μ, thus falling within the size range of New Zealand material, with which they agree in all respects.

Only one blood parasite, *Haemogregarina salariasi* Laird, 1951, has been recorded previously from fishes of the islands north of New Zealand. This species was described from very limited material from one of 13 examples of the blenny, *Salarias periophthalmus*, from the barrier reef near Makuluva Islet, Fiji (6). None of the 28 examples of this fish collected from the New Hebrides, the Solomons, and Fiji during the present studies were parasitized by *H. salariasi*, a small haemogregarine closely resembling *H. bigemina* with which it may ultimately prove to be identical (6, 8). There is increasing evidence of the cosmopolitanism of *H. bigemina*, which is already known from several parts of Europe, Canada, Florida, and possibly South Africa, besides the South Pacific. *Clinus perspicillatus* and *Tripterygion rufopileum* are new hosts for this parasite, which is, however, found in two species of the latter genus in New Zealand. Some taxonomists include *Tripterygion* in the family Blenniidae, and this practice was followed in the earlier account (8). *H. bigemina* is not characterized by close host specificity, having been recorded from the Xenopterygii (family Gobiesocidae) (8) and the Percoidea (family Sciaenidae) (13) as well as from the blennioid families Blenniidae, Clinidae, and Zoarcidae.

Haemogregarina mugili Carini, 1932 (Fig. 2, *b–f*)

All the infections were light ones, the number of parasitized erythrocytes never exceeding one per 10,000. Single endoerythrocytic haemogregarines and a few free forms were the only stages seen.

The forms within red cells (Fig. 2, *c–e*) are crescentic, measuring 8.2 to 10.5 μ by 1.8 to 2.8 μ (av. 9.6 μ by 2.0 μ). Their alveolar cytoplasm stains deep blue with Giemsa and contains numerous tiny granules. The nucleus, which is usually located towards one end (Fig. 2 *c, d*), occupies the full width and about one-third of the length of the body and is composed of intensely staining blocks of chromatin of varying size and shape. The blocks are of irregular outline, and are not comparable with the rounded, subequal, and

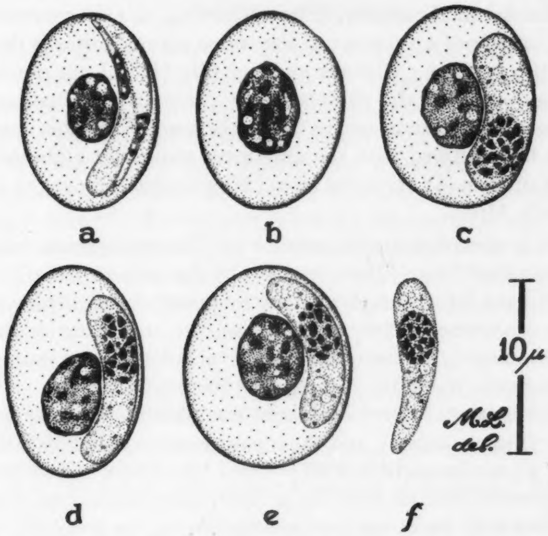

FIG. 2. *a. Haemogregarina bigemina* Laveran and Mesnil. *b.* Uninfected erythrocyte *Awaous ocellaris* (Broussonet). *c–f. Haemogregarina mugili* Carini. All figures ×2550.

progressively more widely scattered, merozoite nuclei seen in the schizonts of *Haemogregarina leptoscopi* Laird, a New Zealand species (7). In the present instance, the masses of chromatin form a more or less oval group, and owe their apparent separation to the fact that the ground material of the nucleus stains only lightly, if at all. This is verified by the examination of overstained examples, in which the nucleus appears reddish-black and homogeneous. Little alteration of the host cell takes place, beyond (usually slight) displacement of the nucleus. Free forms of the organism (Fig. 2, *f*) are crescentic and measure some 10 μ by 2 μ, the nucleus being more compact and homogeneous than that of the endoerythrocytic haemogregarines.

This parasite does not belong to the *bigemina* group of *Haemogregarina*, nor is it referable to the *rovignensis* group, members of which are characterized by a red-staining polar cap at one or both of the extremities of the body (7, 12). It is unlike any of the haemogregarines thus far known from Australian (9) or New Zealand (7, 8) fishes, all of which belong to, or are closely allied to, one or other of these groups.

Some of the known haemogregarines referable to neither the *bigemina* nor the *rovignensis* group are inadequately characterized, and it would be impossible to assign new material to them with any certainty. The occurrence of the present parasite in two species of the family Gobiidae prompted an initial examination of the literature for descriptions of haemogregarines from these fishes. All but one of the species concerned, though, proved to belong to the *bigemina* group. The exception is *Haemogregarina blanchardi* Brumpt and Lebailly, from a European goby (1) which, although of similar size (10 μ by

2.5 μ) to the Guadalcanal parasite, differs in having, as a characteristic feature, a conspicuous vacuole of a diameter of about one micron towards the extremity farthest from the nucleus. Brumpt and Lebailly (1) did not publish illustrations of *H. blanchardi*, nor did they note any peculiarity of nuclear structure. For these reasons, the present species cannot be assigned to this European one. Neither can it be identified with the unnamed, somewhat globular haemogregarine which Fantham (4), recorded from (*Mugil capito* Cuvier) = *Liza ramada* (Risso) in South Africa.

However, it is morphologically similar to *Haemogregarina mugili* Carini, which was described from *Mugil brasiliensis* Agassiz in Brazil. Carini (2) also dealt with light infections, his parasite causing slight displacement of the nucleus of the otherwise scarcely altered host cell, and being characterized by its intensely staining cytoplasm, exhibiting vacuolation and granulation, and a nucleus apparently made up of separated chromatin blocks. The endoerythrocytic and free forms figured by Carini resemble those of the species under discussion very closely indeed, and the measurements of the Brazilian parasite (8 to 9 μ by 2 μ) are compatible with those of the Solomon Islands' one. The case of *H. bigemina* may be cited as evidence that individual species of fish haemogregarines may be of very wide distribution, so geographical isolation need not be held to preclude the identification of these two organisms with one another. The occurrence of both in fishes which commonly frequent estuaries is of considerable interest, as is the parasitizing of species of *Mugil* in both the Atlantic and Pacific. It should be noted, though, that mullets are apt to range widely—*Mugil cephalus* L., for example, occurs in oceanic as well as coastal waters of the South Pacific, Indian, and Atlantic Oceans. At the same time, it must not be overlooked that other oceanic fishes might also carry the parasite, the occurrence of which in *Mugil* sp. and two species of the unrelated family Gobiidae refutes close host specificity as regards this organism.

In the light of these data, the haemogregarine under discussion is identified as *Haemogregarina mugili* Carini, 1932.

The mode of transmission of any of the haemogregarines of fishes has yet to be established. Studies of the Protozoa of New Zealand intertidal zone fishes (8) provided evidence suggesting that questions of the ecology of the environmental niche concerned are as relevant to this problem as is host specificity, for unrelated fishes sharing the same small pool are liable to exhibit the same protozoan parasites. In view of the extreme paucity of records of haemogregarines during these investigations in the South Pacific, the occurrence of five infections (of the seven noted from the total of 1121 fishes) among three of the eight species of estuarine fishes isolated in two small pools by the drying-up of a river points in the same direction. As in New Zealand, however, no blood-feeding invertebrates of groups known to include vectors of haematozoa could be found in these habitats. The only epibionts present on the Guadalcanal hosts of *Haemogregarina mugili* were, as in the case of the New Zealand hosts of *H. bigemina*, trichodinids and other ciliate protozoans. These will be dealt with in a subsequent paper of this series.

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PARASITES OF SOUTH PACIFIC FISHES

II. DIPLECTANUM MELANESIENSIS N. SP., A MONOGENETIC TREMATODE FROM FIJI AND THE NEW HEBRIDES[1]

MARSHALL LAIRD[2]

Abstract

Thirty-six immature examples of the serranid *Epinephelus merra* Bloch were collected from coral pools at Aneityum (New Hebrides) and Makuluva Islet (Fiji), on four occasions from 1952 to 1954. The gills of all of them were infected with *Diplectanum melanesiensis* n. sp., the closest known relatives of which parasitize allied hosts in Japan (*D. epinepheli* Yamaguti) and Celebes (*D. serrani* Yamaguti). The South Pacific species resembles *D. serrani* but differs from *D. epinepheli* in lacking a conspicuous ejaculatory bulb and in having a transversely positioned ovary the elongate terminal portion of which enfolds the right caecum. It is distinguished from both these species by its markedly smaller size (217 to 500 μ by 66 to 117 μ, av., 366 by 92 μ) and the structure of the squamodiscs, which have nine concentric rows of rodlets, the four innermost ones forming complete circlets.

Introduction

The superfamily Gyrodactyloidea (Trematoda: Monogenea) was established by Johnston and Tiegs (1) for that group the members of which lack the usual type of suckers and secure themselves to the host, a marine or fresh-water fish, by means of a posterior haptor armed with hooks. Oviparous gyrodactyloids without either prominent head lappets or a vitello-intestinal duct are referred to the family Dactylogyridae Bychowsky, the subfamily Diplectaninae Monticelli embracing those species having the haptor supplied with two pairs of large hooks, termed anchors, and a pair (dorsal and ventral) of accessory structures, the squamodiscs. Members of this subfamily also exhibit anteriorly directed scale-like spines on the posterior body surfaces.

Sproston (6) assigned five genera to the Diplectaninae: *Diplectanum* Diesing, *Lepidotrema* Johnston and Tiegs, *Lamellodiscus* Johnston and Tiegs, *Squamodiscus* Yamaguti, and *Neodiplectanum* Mizelle and Blatz. The squamodiscs consist of concentric rows of paired lamellae in *Lamellodiscus*, but in the remaining genera are made up of concentric rows of scale-like spines or rodlets, or spine-like hooks, the latter being found only in *Lepidotrema*, which is further characterized by the presence of five haptorial bars, and in *Squamodiscus*. There are only two haptorial bars in *Neodiplectanum*, the intestine of which is a simple lobed sac.

Yamaguti (7) established the genus *Squamodiscus* for species agreeing with *Diplectanum* in having three haptorial bars and a bifurcate intestine, but differing from the latter genus in having the squamodiscs composed of spine-like hooks instead of scale-like spines, and in the absence of an ejaculatory

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Contribution from the Institute of Parasitology, McGill University, Macdonald College, Macdonald College P.O., Que., Canada. The material was collected during a research project initiated by the Royal New Zealand Air Force and supported by a grant from the N.Z. Department of Scientific and Industrial Research.
[2]Institute of Parasitology, McGill University.

bulb. Price (4) regarded *Squamodiscus* as a synonym of *Diplectanum*, and although Yamaguti (8) defended his former position he has since (9) conceded that only *Diplectanum* should stand, feeling, after comparing *D. serrani* Yamaguti, 1953, with *D. epinepheli* Yamaguti, 1938, that "the presence or absence of the ejaculatory bulb as well as the structure of the squamodisc in terms of hooks, rodlets, or scales is of no generic importance." The latter author (9) described two further genera, *Diplectanocotyla*, having a haptorial sucker, and *Pseudolamellodiscus*, having digitiform posterior appendages on the haptor.

Finally, Ramalingam (5) has added a further genus, *Telegamatrix*, to the Diplectaninae, the genitalia being unique in including a copulatory "tentacle" with two-way ducts.

Recognizing only the seven genera *Diplectanum*, *Diplectanocotyla*, *Lepidotrema*, *Lamellodiscus*, *Neodiplectanum*, *Pseudolamellodiscus*, and *Telegamatrix* within the subfamily Diplectaninae, the diagnosis of *Diplectanum* is as follows: Diplectaninae having the haptorial anchors supported by three transverse cuticular bars. Haptor lacking a sucker and digitiform posterior appendages. Squamodiscs usually made up of concentric rows of scale-like spines or rodlets, although sometimes consisting of such rows of spine-like hooks. Cirrus with or without ejaculatory bulb. Copulatory tentacle lacking.

Methods and Material

Young examples of *Epinephelus merra* Bloch are often present in intertidal pools on coral reefs and rocky coasts in the Indo-Pacific area. This fish belongs to the family Serranidae, to which the names of "sea bass" and "grouper" are widely applied. In the Southern Hemisphere serranids are often termed "rock cod", a name given to certain of the Scorpaenidae in the Northern Hemisphere, and desirable in neither case since the fishes concerned are quite unrelated to the true codfishes of the family Gadidae.

Eleven *E. merra* from Aneityum, New Hebrides (August 5, 1952, and March 2–7, 1953), and 25 from Makuluva, an islet near Suva, Fiji (January 2 and February 21, 1954), all proved to have the gills infected with the *Diplectanum* described herein. These fish ranged from 54 to 205 mm. in length, the largest of them being less than half grown.

Haematoxylin-stained gill smears were prepared as described in the first paper of this series (2), and on the second visit to Aneityum some observations were made from life by phase contrast microscopy. The latter technique also proved valuable in the detection of understained structures, such as the vas deferens and the vitelline ducts, in haematoxylin mounts.

Descriptive Account

(Figs. 1–5)

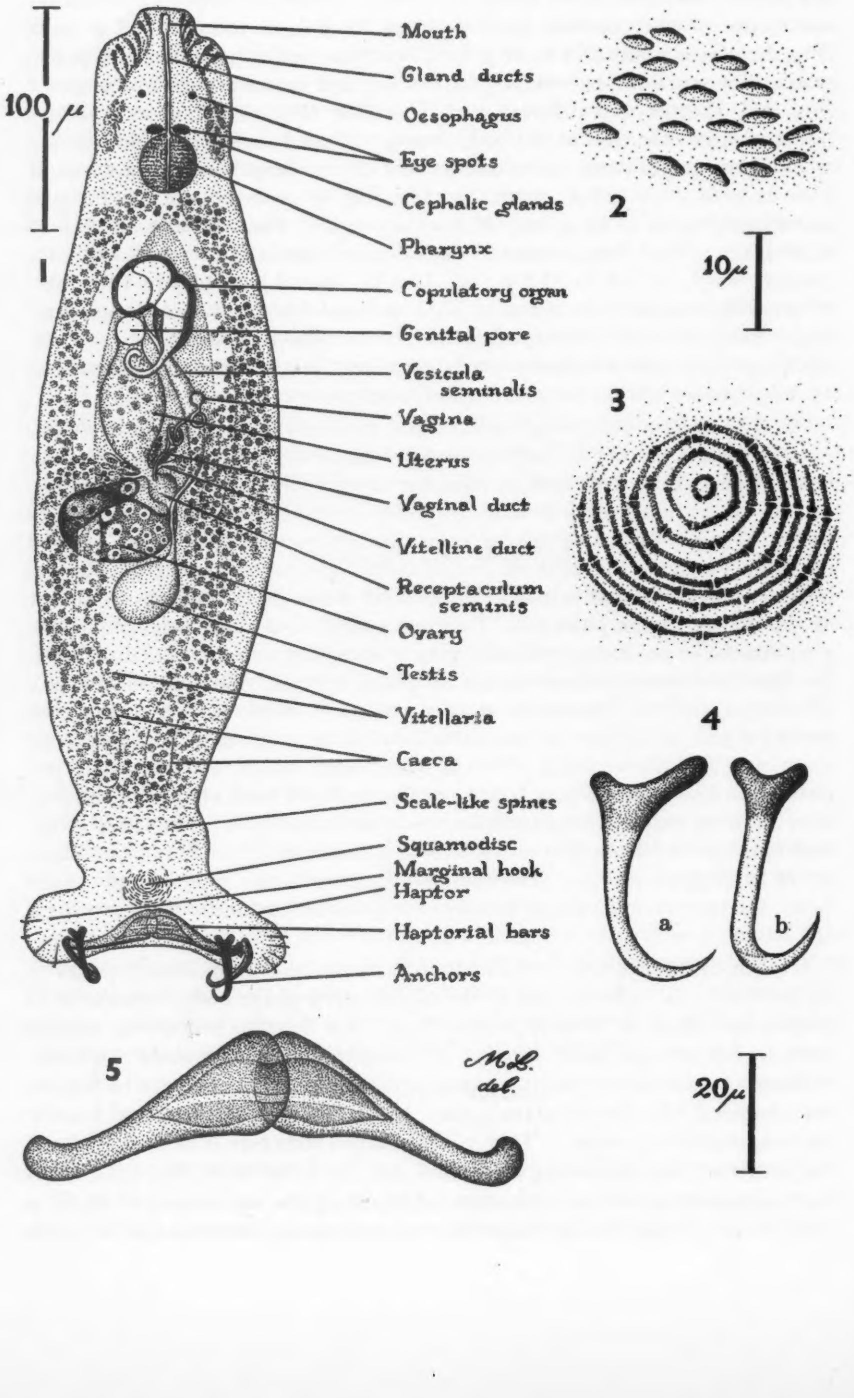
(all averages computed from the measurements of 20 stained specimens)

Body elongate, the length–breadth ratio of living examples ranging from 7:1 at full extension (Fig. 6) to 3:1 at full contraction (Fig. 7), that of fixed

and stained ones (Fig. 1) averaging 4:1. Over-all measurements, 217 to 500 μ (av., 366 μ) by 66 to 117 μ (av., 92μ). The anteriorly directed scale-like spines of the posterior body surfaces 1.8 μ high and 3 to 4.2 μ wide (Fig. 2). Squamodiscs 24 to 30 μ by 22 to 28 μ, made up of nine concentric rows of elongate dumbbell-shaped rodlets, the four innermost circlets complete (Fig. 3). Haptor well differentiated, its width (104 to 127 μ, av., 112 μ) rather greater than that of the body proper. Dorsal anchor (Fig. 4*a*) having an elongate ventral root and a nodular dorsal one, length to top of curve of hook 36 to 44 μ (av., 39 μ), ventral anchor (Fig. 4*b*) with roots of equal length and measuring 34 to 41 μ (av., 36.5 μ) in length. Central haptorial bar 30 to 45 μ (av., 41 μ) long, narrowed medially and expanding on each side to a greatest width of 10.5 to 13.5 μ (av., 11.5 μ), lateral bars 14.5 to 19 μ (av., 16 μ) wide basally, narrowing to a curved and bluntly rounded tip, their length being 40 to 53 μ (av., 45 μ) (Fig. 5). Marginal hooks, or hooklets, 8 to 9.5 μ long; full complement not determined, but Sproston (6) stated that 14 are probably always present in the Diplectaninae.

Head 53 to 69 μ (av., 62 μ) wide at base, two distinct lateral lobes and two anterior ones apparent in fully extended living examples (Fig. 6). All four lobes margining the truncate anterior end of fully contracted specimens (Fig. 7), the central cleft clearly leading to the ventrally located mouth. It is seldom possible to distinguish between the two anterior head lobes in fixed material (Fig. 1). The cephalic glands (sometimes termed sticky glands) are laterally positioned and extend from the level of the anterior eye spots to that of the middle of the pharynx. They are linked with three pairs of swollen gland ducts, or head organs, discharging towards the anterolateral margins of the head and clearly visible in life by phase contrast illumination (Fig. 6). The two anterior eye spots are smaller and more widely separated than the posterior pair, which lie one on either side of the esophagus at the anterior margin of the pharynx (Fig. 1). Although both mouth and esophagus are difficult to make out in stained specimens by ordinary bright field illumination, they show up well by phase contrast, as does the pharynx. The latter structure is round to elliptical in surface view, measuring 27 to 35 μ (av., 29.5 μ) by 26 to 29 μ (av., 27 μ). Proceeding back from it, one on either side of the body, the two caeca terminate blindly near the posterior constriction preceding the haptor.

Oval testis transversely positioned across the mid-line immediately posterior to the ovary, towards the rear of the middle third of the body, measuring 28 to 45 μ (av., 36 μ) by 22 to 32 μ (av., 26 μ). Vas deferens proceeding forward from its left anterior limit, forming an elongate-fusiform vesicula seminalis. Although an ejaculatory bulb could not be detected one may perhaps be present but obscured, like the ejaculatory duct, by the very conspicuous and heavily staining copulatory organ. This comma-shaped structure is located at about the anterior third, measuring 34 to 46 μ (av., 38 μ) by 30 to 35 μ (av., 33 μ) in its greatest diameters. Its total length along the mid-line is 49 to 58 μ (av., 52 μ). Septa divide it into four compartments, from the last of which


100 μ
1
Mouth
Gland ducts
Oesophagus
Eye spots
Cephalic glands
Pharynx
Copulatory organ
Genital pore
Vesicula seminalis
Vagina
Uterus
Vaginal duct
Vitelline duct
Receptaculum seminis
Ovary
Testis
Vitellaria
Caeca
Scale-like spines
Squamodisc
Marginal hook
Haptor
Haptorial bars
Anchors
2
10μ
3
4
a
b
5
M.L. del.
20μ

the thin-walled, tubular cirrus proper curves towards a common genital pore located immediately behind the first compartment of the copulatory organ and to the right of the mid-line. Dorsally situated prostatic cells lie in the field between the copulatory organ and the ovary.

Ovary transversely positioned, appearing broadly oval on casual examination and measuring 41 to 56 μ (av., 45 μ) by 16 to 31 μ (av., 23 μ) in its greatest diameters. Closer examination discloses a narrow and elongate distal portion which curves around the right caecum and proceeds back towards the mid-line just beneath the ventral surface of the body (Fig. 1), the over-all length of the ovary along the mid-line being 66 to 95 μ (av., 80 μ). Swollen uterus receiving the vitelline ducts and receptaculum seminis at its posterior end, and proceeding anteriorly to the right of the mid-line. Vaginal opening to the left of the mid-line, near the level of the middle of the uterus, the duct narrow and convoluted. Vitellaria extending from the level of the posterior limit of the pharynx to just beyond the caecal ends.

Discussion

Only two species of *Diplectanum* have previously been recorded from Australasia. Both of them were described from the gills of Queensland fishes (1), *D. fluviatile** (Johnston and Tiegs) parasitizing the fresh-water golden perch, *Plectroplites ambiguus* Richardson, and *D. girellae* (Johnston and Tiegs) infesting the marine black bream, *Girella tricuspidata* Quoy and Gaimard. These species measure 950 by 260 μ and 700 by 160 μ respectively (1), and are thus much larger than the parasite of *Epinephelus merra*, from which they also differ in the morphology of the genital organs and in the structure of the squamodiscs. The latter are composed of 25 rows of rodlets in *D. fluviatile* and of about 15 in *D. girellae*, none of the rows forming complete circlets in either case.

In accord with the generalization that monogenetic trematodes tend to exhibit high host specificity in nature (3), closely related fishes being parasitized by the same or closely related species of Monogenea, the affinities of the present species lie with two gill parasites of other members of the family to which the host belongs, the Serranidae. Yamaguti (8) described *D. epinepheli* from *Epinephelus akaara* (Temminck and Schlegel) from the Inland Sea, Japan, and subsequently (9) described *D. serrani* from *Serranus* sp. from Celebes. Both species are appreciably larger than the one dealt with herein, measuring 450 to 800 μ by 130 to 160 μ and 450 to 660 μ by 100 to 140 μ respectively.

*Termination of specific name emended by Sproston (6). Originally *Lepidotes fluviatilus* Johnston and Tiegs, then *Diplectanum fluviatilis* (J. and T.) Price.

FIGS. 1–5. *Diplectanum melanesiensis* n.sp. All figures prepared with the aid of a Zeiss-Winkel drawing apparatus, from haematoxylin-stained slides.
FIG. 1. Whole animal, ventral view. ×330
FIG. 2. Scale-like spines of posterior part of body. ×1425
FIG. 3. Squamodisc. ×1425
FIG. 4. (*a*) Dorsal and (*b*) ventral anchors. ×875
FIG. 5. Haptorial bars. ×875

The squamodiscs of *D. epinepheli* are relatively very large, measuring 80 by 60 μ and extending across the full width of the animal at the junction of the body proper and haptor. They are made up of about 16 rows of rodlets, the anterior ones of which are concentric (although none of them form complete circlets) while the posterior ones are straight or only slightly curved (8). This species differs further from the present one in its elongate pharynx, the longitudinal alignment of testis and ovary, and the presence of a muscular ejaculatory bulb.

D. serrani stands closest to the parasite of *Epinephelus merra* in general morphology as well as in over-all size. Neither animal has a conspicuous ejaculatory bulb, and in both cases the ovary is transversely positioned and has an elongate terminal portion which enfolds the right caecum. The haptorial structures compare closely in several respects, notably in the size and shape of the anchors and the relatively small size of the squamodiscs. The latter measure 39 to 45 μ by 33 to 42 μ in the case of *D. serrani* occupying about one-half of the body width at the posterior constriction, while those of the South Pacific species measure 24 to 30 μ by 22 to 28 μ and occupy about one-third of the body width at this location. There are from nine to 11 rows of rodlets in the squamodiscs of *D. serrani*, none of them, however, forming complete circlets. Other features distinguishing the Celebes parasite from the one under consideration are the shorter esophagus and more elongate pharynx of the former, and the longitudinal alignment of its testis.

Differing as it does from other members of its genus the parasite of *Epinephelus merra* described herein is regarded as a new species, for which the name of *Diplectanum melanesiensis* n.sp. is proposed, the specific name referring to its occurrence in two of the island groups of Melanesia. The type slide has been deposited in the Helminthological Collection of the United States National Museum, and a paratype in the collection of the Dominion Museum, Wellington, New Zealand.

Acknowledgments

Thanks are due to Mr. A. C. Stevenson, who provided accommodation during the two visits to Aneityum, to Professor Satyu Yamaguti of Okayama University for his kindness in providing a typescript copy of his description of *Diplectanum serrani*, and to my wife for her help on all the field trips and for the photomicrographs reproduced in this paper.

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NOTE: Figs. 6, 7 follow.

FIG. 6

FIG. 7

FIGS. 6 AND 7. Living example of *Diplectanum melanesiensis* n.sp. in fully extended (Fig. 6) and fully contracted (Fig. 7) states. Phase contrast photomicrographs by Elizabeth Laird, from material collected at Aneityum, February, 1953.

STUDIES OF THE FEEDING ORIENTATION OF THE JACK PINE SAWFLY, NEODIPRION PRATTI BANKSIANAE ROH.[1]

A. W. GHENT[2]

Abstract

The heads of feeding larvae of the jack pine sawfly are consistently oriented towards the needle tips. This orientation is not obtained from the apically-directed needle teeth, and is found to be independent of gravity and of the flexibility of the foliage. Though light can act to disrupt the orientation, larvae adopt the orientation in darkness so that light cannot be postulated as an essential directive stimulus. The behavior is identified as a "free-end" response, and possible underlying mechanisms are discussed.

Introduction

Larvae of the jack pine sawfly, like the larvae of related sawflies on other coniferous hosts, consistently orient themselves while feeding so that their heads are directed toward the needle tips. They feed in groups of two, three, or more individuals aligned side-by-side and parallel to the long axis of the needle, as illustrated by Fig. 1. During the first and second instars the larvae skeletonize the foliage, whereas the more advanced larvae consume the foliage in its entirety; throughout larval life, however, the same feeding orientation is maintained. From a feeding site initiated at or near the needle tip the larvae eat their way back towards the shoot, leaving only a small stump of needle tissue at the basal sheath. Thus the feeding orientation is efficient, allowing virtually complete utilization of the available foliage; and safe, since it places the insects in no jeopardy of severing their attachment to the host tree. The advantages of the behavior then are such that one is not prompted to wonder at its persistence through selection; the more challenging problem is presented by the mechanisms of response governing its operation.

Influence of Needle Structure

Needles of the jack pine, *Pinus banksiana* Lamb., like the needles of other pines, bear minute apically-directed teeth along their edges. It seemed logical to suppose, as a starting point, that the larvae might obtain their orientation from the orientation of these teeth. A feeding orientation derived in this way should be adopted even on needles detached from the shoot, so that to test this possibility individual needles were placed in separate cork-stoppered vials along with a single fourth- or fifth-instar larva. The basal end of each needle was dipped in molten wax, so that odors emanating from the broken tissue

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Contribution No. 431, Forest Biology Division, Science Service, Department of Agriculture, Ottawa, Canada. Based in part on data included in a thesis submitted to the University of Toronto in partial fulfillment of the requirements for the degree of Master of Arts. These data were gathered during studies conducted at the Department of Zoology, University of Toronto, and supported by a fellowship donated by the Spruce Falls Power and Paper Co., and by a Research Council of Ontario scholarship.
[2]Forest Insect Laboratory, Sault Ste. Marie, Ontario.

would not attract the larvae to the basal end. Possible influences of the paraffin were then equated by dipping the apical end of each needle as well, taking care not to overheat the wax since this can destroy the acceptability of the foliage to the larvae. Possible differences in directive effects of the cork stopper and the glass surface of the opposite end of the vial were equalized by reversing the orientation of alternate needles within the vials. No consistent orientation could be given to the larvae on admission to the vials, but since most larvae explored the vial for some minutes before feeding this difficulty seemed not to be critical. Finally the vials were laid horizontally on a tray and covered with a double thickness of black cloth, a precaution taken to eliminate directive effects of light fixtures in the laboratory. The experiment was repeated four times, and for each experiment 44 vials were prepared. The same 44 larvae were used in each repetition of the experiment, some larvae feeding not at all and others feeding only once or twice during the four repetitions. Brief examinations of the vials were made at 10-minute intervals, the black cloth, of necessity, being removed for a few moments during the examination. It was found to be more economical of time to re-stage the experiment after about half of the insects had begun to feed than to wait for the remainder.

The results of the four repetitions of this experiment are given in Table I, along with relevant figures from the chi-square tests of the hypothesis of a 1:1 ratio of basal and apical orientation. These tests merely bear out what is apparent from the raw data without recourse to analysis: that there is no consistent trend in either direction. It is concluded that jack pine sawfly larvae are unable to derive their normal orientation from the teeth of detached needles.

TABLE I

TEST OF THE HYPOTHESIS OF A 1:1 RATIO OF BASAL TO APICAL ORIENTATION ON DETACHED NEEDLES

Experiment No.	Orientating towards		Expected 1:1	Chi square	Degrees of freedom
	Apex	Base			
1	10	5	7.5:7.5	1.67	1
2	11	10	10.5:10.5	0.05	1
3	10	21	15.5:15.5		
				3.90*	1
				0.80	1
4	8	12	10.0:10.0		
	39	48	43.5:43.5		
			Total chi-square	6.42	4
			Chi-square of total	0.93	1
			Heterogeneity chi-square	5.49	3 Not significant

*Expt. No. 3 falls just beyond the .05 level of significance with 1 degree of freedom. In consideration of the other data, there is no reason to suppose that this represents other than a random departure.

FIG. 1. Typical feeding group of jack pine sawfly larvae, illustrating the characteristic orientation towards the needle tip. Insects shown are early second-instar larvae, skeletonizing a needle.

Larvae that fed more than once during the four repetitions of this experiment provided data on the orientation adopted on detached needles at successive feedings. On superficial examination there appeared to be a weak correlation between the orientation at one feeding and the orientation at a successive feeding. Contingency analysis failed to bear out the significance of this apparent tendency, however, and the question was not pursued.

The next possibility followed as an outgrowth of the first, for if larvae could not adopt their normal orientation on detached needles, it might then be of little consequence which end of the needle was attached to the shoot. If jack pine needles are gently plucked from the basal sheath, one at a time, the basal sheath itself will usually remain attached to the shoot. The basal sheath then provides a convenient cup into which a single needle may be re-inserted, tip foremost, and secured with a drop of molten paraffin. In this way it is possible in a few minutes to reconstruct a shoot bearing 40 to 50 needles, all of which are attached by what are actually their apical ends.

Again in this experiment both ends of the needles were dipped in wax to equate possible directive influences from this source. The experiment was repeated eight times, using 10 insects in each instance. It was performed in complete darkness, and in normal room light, and larvae from the full range of instars were tested. The experiment was started by placing 10 insects at the top of the reconstructed shoot, from which point they were free to wander over the foliage until they began to feed. Individual larvae would often wander off the foliage and on to the glass lantern globes that enclosed the shoots, so that the least successful of the eight experiments offered observation of only three insects, and the most successful, observation of nine.

Forty-one larvae were observed to feed during the eight experiments, and in every instance the orientation adopted was towards the new "tip" of the needle, which was of course the actual base. The experiment in which only three larvae fed was unfortunately the only experiment in which first-instar larvae were used, but these three larvae also oriented towards the new "tip" of the needle. This instar was possibly the most critical, for if the larvae were to be influenced by the marginal teeth, they should have been so influenced when the size of the teeth was greatest in proportion to body size of the insects. It is concluded that the larvae of this species will adopt a feeding orientation *towards the free end* of the needle, normally the natural apical end, but independent of any structural peculiarities of the apical end that are not also shared by the basal end when this is presented as the free end of the needle.

It is satisfying to find that larvae of the jack pine sawfly do not depend upon the needle teeth for their feeding orientation, for the larvae of the balsam fir sawfly, *N. abietis* Harr., manage to adopt the same orientation on the smooth-edged needles of balsam fir, *Abies balsamea* (L.) Mill. Furniss and Dowden (1) report that this same orientation is adopted by *N. tsugae* Midd. on the foliage of western hemlock, *Tsuga heterophylla* (Rafn.) Sarg. Pine sawflies reared at the Forest Insect Laboratory have included *N. swainei* Midd., *N. lecontei* (Fitch), *N. pinetum* (Nort.), *N. sertifer* (Geoff.), *N. nanulus* Schedl,

and *N. virginianus* Roh., and all of these adopt this same feeding orientation. It seems reasonable to postulate a common set of response mechanisms governing feeding orientation throughout the genus.

Influence of Gravity

The possibility that the feeding orientation might represent a response to gravity was never seriously entertained, for the orientation is adopted in the field on needles growing in all directions from shoots which in turn stand at a wide range of angles with respect to gravity. It was, however, a simple matter to eliminate gravity in a more formal way. A jack pine shoot on which the needles stood closely appressed to the stem was placed apex-foremost into the water-jar base of a lantern-globe rearing container, so that the needles were directed for the most part straight down. A feeding colony of about 40 third- and fourth-instar larvae was transferred to this foliage, which they completely consumed by the end of the following day. Feeding began as usual at the needle apices, the larvae pointing straight downwards and working their way back up the needles against the pull of gravity as they fed.

Influence of Attachment

The experiments so far reported pointed to *attachment* as the critical circumstance to which the larvae were responding, and it was soon apparent that it mattered not at all to what object the needle was attached: larvae oriented towards the free end of needles attached to glass slides, to cork stoppers, and in one instance—to the laboratory bench. Efforts to isolate the qualities given to a needle by *attachment* led to the pursuit of a series of simple experimental designs, two of which will serve to illustrate the principles disclosed by these studies.

The first of these was prompted by the notion that the larvae might obtain their orientation from some minute flexure of the needles under their own weight. The experiment involved setting the entire length of a needle, to about one-third its width, edge-on into the face of a flat pool of molten paraffin just as it solidified. The attached end of a needle normally disappears into the basal sheath, presenting no apex-like angularity. This quality of attachment was mimicked by pouring a second and smaller pool of paraffin over one end of the needle—in this experiment the apical end. Four larvae initiated feeding sites under these conditions, all at the exposed basal end. Two of the four larvae mounted the needle lengthwise in the usual way, but the other two fed from positions on the surface of the paraffin almost at right angles to their needles. Only the head capsules of these latter larvae were turned into the customary orientation; back of the head capsules their bodies turned abruptly so that the metathoracic legs and the abdominal prolegs were in contact with the paraffin in which the needles were embedded. The experiment illustrates first of all that needle flexure is not an essential directive stimulus, and secondly that an exposed corner or angle at the end of a needle will prompt the larvae to begin feeding at that point. There is nothing in the rigidity of attachment that is responsible for the orientation: in another

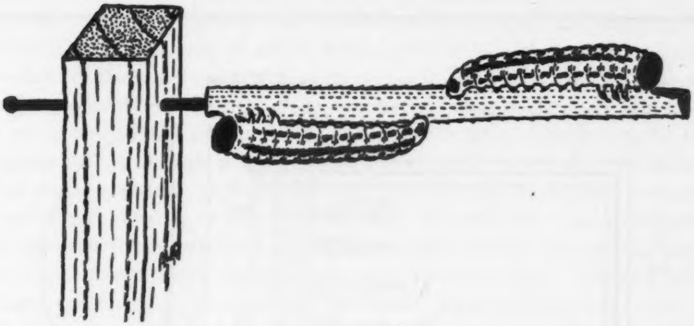

FIG. 2. Orientation adopted by two late-instar larvae on a needle mounted so that the angularity of both ends was available for the initiation of feeding sites.

experiment it was found that larvae began feeding at either end of needles attached at their mid-points to the ends of thin wooden dowels, just as they fed at either end of completely detached needles. In addition, this experiment with embedded needles indicates that even the natural orientation parallel to the needle axis is not an essential precondition for triggering the feeding response, but that, in the absence of another surface such as that provided by the pool of paraffin, it is simply the inevitable orientation imposed by the geometry of the undisturbed environment.

The second experiment provided the most unequivocal results with the observation of only two feeding insects, both of which were presented with the same needle as illustrated in Fig. 2. Basal and apical ends of this needle were removed with a razor blade, and the cut ends sealed with paraffin. The needle was then attached at its basal end to a balsa wood pillar with a fine insect pin, so that both the attached end and the free end presented sharp angles at which feeding might begin. Ten larvae were placed on the pillar, and the apparatus was covered with a glass jar and black paper covering. After 15 minutes the coverings were removed, and two larvae were found to have initiated feeding sites, one at either end of the needle, as illustrated. It is clear that contrasts in rigidity and flexibility at the attached and the free ends, if such exist, have no bearing on the feeding orientation, and that the free end owes its attractiveness to the fact that it is an *end*. It seems most probable that this attractiveness is related to the mechanical advantage of biting against an *angle* or *corner*, as opposed to biting into a straight edge or flat surface.

Influence of Light

The possibility that visible light might be an important directive stimulus in the feeding orientation was eliminated by the repeated observation that experiments of other designs could be conducted either in complete darkness or under the normal laboratory light conditions without affecting the response to the free end of the needle. At the same time it can be shown that the photopositive response of this species, described by Green (2), will take precedence over the "free-end" response here described.

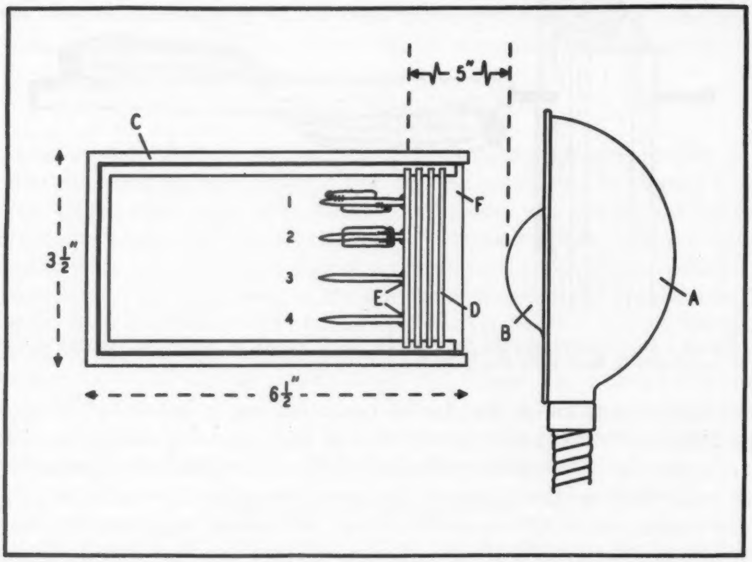

FIG. 3. Orientation adopted by the five larvae observed feeding in Expt. No. 5, Table II. A: gooseneck reflector lamp; B: 40 w. frosted bulb; C: slide box light chamber, as described in text; D: glass slides; E: paraffin wax, used to secure the needles to the glass slide; F: glass plate fitted to rest on slide spacers, so that the lid might be removed.

A simple light chamber, illustrated in Fig. 3, was constructed from a small wooden slide box of the sort employed to store a single row of microscope slides. The inside of the slide box was covered with several coats of "Blackboard Black" non-gloss paint, and fitted with a plate glass covering which rested on top of the small wooden teeth provided as spacers between the slides. One end of the box was removed, and the first four slots at this end fitted with glass slides to provide a series of three dead-air spaces to insulate the interior of the box. The regular lid of the slide box, also painted black, was used to prevent light entering the box except at the open end. This lid rested on top of the plate glass covering already described, an arrangement that kept the larvae confined even when the wooden lid was removed during periodic examinations. Needles were attached to the innermost glass slide with a small drop of paraffin, which, after cooling, was whittled down to the smallest amount sufficient to secure the needles. In this way four or five regularly spaced needles could be attached perpendicular to the surface of the slide, so that they stood out horizontally and parallel to the long axis of the slide box.

In each experiment 10 fourth- or fifth-instar larvae were placed in the center of the box, from which point they were free to find the needles and adopt an orientation without operator bias. As a technical convenience the larvae were starved for 24 hours before the experiments, since preliminary trials indicated that starved larvae would seek out the needles more promptly.

Under the various circumstances summarized in Table II the experiment was repeated eight times, four of which were in the nature of control experiments. In the first of these a double wrapping of black cloth was used to ensure complete darkness within the box, the experiment being performed in a dimly-lit room as an additional precaution. Five insects were observed to be feeding during the brief intervals when the black cloth and the lid were removed, and each of these was seen to have adopted the normal orientation towards the free end of the needle, in this instance the unwaxed tip. Experiments 2, 3, and 4 were conducted without the black wrapper, allowing normal room light to enter the box through the glass slides at the open end. These conditions presented the larvae with a weakly "reversed" light gradient, stronger at the attached than at the free end. Neither this nor the other experimental variations—attachment at apical and basal ends and the presence or absence of paraffin at the free end—affected the results, for all 20 of the larvae observed feeding during these experiments oriented toward the free ends of the needles.

For the remaining experiments a 40-w. frosted bulb in a gooseneck reflector lamp was placed approximately five inches from the glass slide to which the needles were attached, the reflector being directed towards the box as illustrated in Fig. 3. Again the end of attachment and the treatment of the free end were varied. In each experiment, to a greater or lesser degree, the presence of a strong reversed light gradient disrupted the uniformity of orientation among the feeding insects. A few maintained the characteristic orientation toward the free end of the needle, but the majority in all four experiments faced the attached end. Oriented in this manner, a single larva or a group of two or three would quickly chew their way through the needle until it was completely severed. The short drop to the bottom of the slide box seemed not to disturb the larvae, for they would continue to feed lying now on their backs or sides. In this fashion the insects themselves no longer moved, but the

TABLE II

SUMMARY OF THE RESULTS OF EIGHT EXPERIMENTS PERFORMED WITH THE SLIDE-BOX LIGHT CHAMBER ILLUSTRATED IN FIG. 3

Expt. No.	No. needles	Attached end	Free end	Exptl. conditions	No. orientating towards: Free	Attached
1	4	Base	Not waxed	A	5	—
2	4	Base	Not waxed	B	7	—
3	5	Tip	Not waxed	B	7	—
4	5	Tip	Waxed	B	6	—
5	4	Base	Not waxed	C	1	4
6	4	Base	Not waxed	C	1	5
7	4	Base	Not waxed	C	2	4
8	5	Tip	Waxed	C	4	6

EXPERIMENTAL CONDITIONS: Ten IV or V instar larvae in each experiment, some of which did not feed in all experiments except No. 8.

A. Complete darkness.

B. Normal room lighting.

C. 40 w. frosted bulb directed into the light chamber, as illustrated in Fig. 3.

same retrograde crawling movements served, as feeding progressed, to pull the needles up between their legs until the foliage was as usual completely consumed.

Figure 3 illustrates in particular the results obtained in Expt. No. 5. The pair of insects feeding on Needle No. 1 is of especial interest, for these faced in opposite directions, holding on to opposite edges of the needle. A similar instance was observed in Expt. No. 8, in which four larvae formed two groups of two, the groups orienting in opposite directions. Thus the orientation adopted under these conditions seems to depend on the particular larva: perhaps depending on the feeding history of the individual, or on the chance selection of needles more exactly perpendicular to the light source, for on such needles the shadow of their own bodies might prevent the disruption of the normal response to the free end of the needle. In any case it is apparent that under some circumstances the photopositive response will take precendence over everything except the underlying behavior of feeding on available foliage. Thus there emerges a clear-cut example of a hierarchical order of independent behaviorisms, their independence documented by their liability to experimental rearrangement in bizarre combinations.

It is probable that under most natural circumstances the direction towards the needle tip will appear brighter to a larva than that towards the base, darkened as this latter aspect must be by the shoot itself and by the converging needles. The influence of light may then be looked upon as enforcing the "free-end" response under natural conditions: the normal light gradient present on jack pine foliage serving to attract larvae to the needle tips, where the "free-end" response may operate.

Discussion and Conclusions

Throughout the latter part of this paper the mechanism of orientation has been described as a "free-end" response. This terminology has been used to avoid the implication that the needle apex possesses any structural virtue other than the fact that it is not attached to the shoot. Larvae appear to orient towards the apical end because it is accessible to biting, adopting the basal end with equal consistency when this is presented as the more accessible, and adopting both ends when both are accessible.

Under natural conditions larvae of the advanced instars seem invariably to initiate their feeding sites no more than a millimeter or so from the extreme apical point of the needle. For this reason I would be completely satisfied with the results presented on the influence of attachment, were it not for the high incidence of feeding-site establishment by first- and second-instar larvae at points 10 to 15 mm. or more from the needle tips. The larvae in Fig. 1 for example began feeding at a point nearly 10 mm. from the needle tip. On several occasions, however, I have observed first-instar larvae to go to the extreme apex of the needles, and then to work backwards as if "testing" for a point at which easy access to the tissue might be gained. It may be that feeding sites initiated some distance from the needle tip are in every case initiated by larvae that begin by "testing" the foliage at the extreme apex,

and obtain their feeding orientation at that point. The possibility remains, however, that some of these mid-needle feeding sites may not have this as their explanation, so that until more positive experimental evidence is obtained it is wiser to regard our understanding of the feeding orientation responses as incomplete. At the same time the experiments so far performed may be said with confidence to have identified a "free-end" response, which we may accept at the same level of generality at which we accept a phototactic response—without further understanding of the complex of neural and muscular reactions responsible for the actual movement. In the case of the "free-end" response, the underlying mechanism seems to be related to the problem of biting into the tough needle tissue: the explanation that this is best accomplished by biting against the angularity of a free end neatly encompasses all observations on the points of initiation of feeding sites by the later instars, and may prove capable of extension without any fundamental change in principle to include the mid-needle feeding sites of many first- and second-instar larvae.

The explanation is satisfying as it stands, since the mechanism of orientation proposed is neither more nor less complex than it need be to act with complete efficiency under natural circumstances. The species appears to have no orientation response *as such*: it begins feeding where it can, and the rest is dictated by the geometry of its environment. Faced with more succulent foliage some additional safeguards might have been necessary, but the ease with which the orientation may be experimentally disrupted by an unusual light gradient, or bizarre needle attachment, demonstrates that no safeguards are present that are not required: a limitation characteristic of "instinctive" behavior.

The mechanisms of response governing the orientation of larvae that eat whole leaves may be studied with a multitude of insect species on as many hosts: the directive value of the leaf venation of broad-leafed plants offers a possible avenue of rewarding experimentation. The value of any such study lies not only in the particular problem of behavior it may clarify, but in the insight we may gain into the balance and interplay of intelligent awareness of circumstance on the one hand, and the pursuit of blind response on the other insofar as the emphasis has shifted with specialization along the various pathways of evolution

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STUDIES ON STRONGYLOIDES OF PRIMATES

II. FACTORS DETERMINING THE "DIRECT" AND THE "INDIRECT" MODE OF LIFE[1]

PREMVATI[2]

Abstract

Under optimum conditions *Strongyloides fülleborni* has an indirect life cycle but under adverse conditions, only infective larvae and males are developed. Adverse factors such as pH and consistency of media, absence of food, and unsuitable temperatures appear to act directly on the first-stage rhabditiform larvae developed from the eggs of parasitic females. The potential female larva alone becomes an infective larva, the potential male being unaffected. There is a single free-living generation and all eggs laid by free-living females develop directly into infective larvae. The infective larvae developed directly or indirectly can develop only into parthenogenetic females.

The material used in this study was obtained from the viscera of 1500 rhesus monkeys from India, dissected over a period of 9 months at the Institute of Parasitology. The intestinal contents only were used for all the experiments in order to avoid contamination from free-living nematodes.

The genus *Strongyloides* has two distinct types of development outside the host body. A direct or homogonic is one in which the eggs of the parasitic female hatch into rhabditiform larvae which metamorphose directly into infective or filariform larvae. The indirect or heterogonic type is that where the eggs of the parasitic female give rise to rhabditiform larvae which metamorphose into free-living adults; these in turn, after fertilization, lay eggs from which hatch rhabditiform larvae which develop into infective larvae only. In other words, there is an alternation of generations. The possible cause of this phenomenon has been the subject of much controversy.

Leuckart (24) suggested the possibility of two strains, one having only a parasitic generation and a direct mode of life, and the other having both a parasitic and a free-living generation.

Braun (2) demonstrated that environmental factors such as temperature, humidity, composition of faeces, etc. do not determine the method of development.

Leichtenstern (22, 23) followed Leuckart in suspecting two strains and believed that an indirect life cycle is predominant in the tropics and a direct one in temperate regions.

Darling (7) believed the female to have two types of progeny, those expelled into crypts and those embedded in the intestinal wall. He believed that the intraepithelial cell-developed larvae furnish the direct phase and that those expelled directly become indirect phase larvae.

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Contribution from the Institute of Parasitology, McGill University, Macdonald College, Macdonald College P.O., Que., Canada, with financial assistance from the National Research Council of Canada.

[2] Margaret McWilliam Fellow (awarded by Canadian Federation of University Women). At present on leave from the Department of Zoology, University of Lucknow, Lucknow, India.

Sandground (26) was of the opinion that the parasitic female is not parthenogenetic but syngonic and that fertilized eggs give rise to indirect and unfertilized eggs to direct development.

Nishigori (25) observed that if young larvae passed with the faeces meet favorable conditions such as nutriment, humidity, temperature, and oxygen, they develop to sexually mature adults, the eggs of which develop into infective larvae.

Faust (8, 9) noted that if patients infected with *Strongyloides* undergo treatment with gentian violet, the development is always direct regardless of the previous mode of development. After the recovery of what he believed to be parasitic males, Faust (10) suggested that fertilized eggs produce heterogonic development and that unfertilized eggs result in homogonic progeny. Faust (11) stated that:

> "The fundamental, basic mode of development is indirect. The direct type has been developed as a result of unfavourable environmental conditions, and the hyperinfective type as an adaptation to purely parasitic life within the host."

Beach (1) stated he could influence the mode of development by changing the nutritional status of the culture media. He concluded that as nutritional conditions become less and less favorable more and more larvae undergo direct development.

Kreis (21) claimed that in the direct life cycle the parasitic female is accompanied by a male, while the parasitic female of the indirect type is parthenogenetic.

While working with infections produced by a single larva of *Strongyloides ratti*, Graham (16) states:

> "Analysis of the data from single larva infections indicates that a male parasite is unnecessary in the bionomics of *S. ratti* either to maintain parasitic fertility or to explain the two modes of larval development."

Chitwood and Graham (6) demonstrated cytologically the absence of a vitelline membrane on developing eggs in parasitic females of *S. ratti* which could not, therefore, be fertilized. They believe the parasitic female to be parthenogenetic. Graham (17, 18, 19) found marked differences in the life cycle of *S. ratti*, resulting in pure lines of homogonic and of heterogonic types. Each type of infection produces progeny predominantly of its own type of development. He also showed the existence of a seasonal variation; in spring and summer the development is predominantly heterogonic, while in winter there is a considerable drop in the percentage of indirect types. In the case of *S. ratti* he explained this phenomenon as follows:

> "The rat responds physiologically to changing meteorological conditions and that the altered environment thus produced leads to changes in the relative frequency with which progeny of heterogonic

development are produced. This modified productivity of *S. ratti* is viewed as being mediated through the agency of the host and not as a result of a changed environment encountered by the young larvae outside the host's body."

He concluded, therefore, that the mode of larval development of *S. ratti* progeny is determined prior to oviposition.

Brumpt (3) showed that *S. papillosus* of sheep has a predominantly direct development but when rabbits are infected with the sheep strain the progeny has a predominantly indirect development.

Sandground (26) after his experiments with *S. stercoralis*, *S. fülleborni*, and *S. papillosus* stated:

"Infection of certain abnormal hosts leads to changes in the life cycle, and the suggestion is made that direct development in certain species originally arose following the introduction of the more primitive heterogonic parasites into new species of hosts."

In 1933, Faust and Kagy (12) concluded their observations by stating:

"Repeated passage of pure type human strains through canine hosts invariably produced alterations. These tended to simplify themselves into the direct type of development. Passage of the Chimpanzee strain through dogs failed to modify it from the original indirect type, but in man the stability was reduced. The organism bred true to type in the macaque but after this passage was unable to develop in dogs. Change from the hyperinfective and indirect types to the direct types was accompanied by a decrease in the virulence of the organism and increased failure to establish itself in the canine host."

Griffiths (20) made serial transfers of *S. agouti* into an experimental host—the guinea pig—and came to the conclusion that a mixed type (free males and filariform larvae) of development occurs which at times changes to indirect development similar to that found in the agouti. He further got a reversion to the indirect mode of life when he cultured in sterile agouti faeces the ova from faeces of guinea pigs infected with *S. agouti*.

Galliard (13, 14, 15) concluded that different biological and geographical races of *S. stercoralis* exist, distinguishable only by their degree of infectivity to the dog, and that this fact accounts for the diversity of opinion on the identity of *Strongyloides* of man and dog. He also found that not only was the virulence lessened by first keeping the larvae at 8° C. for some days and then at 30° C. for several hours, but that the proportion of direct cycles increased until only direct development occurred. By treating infected dogs with x rays, the infectivity of larvae in subsequent passages was lessened and the proportion with a direct cycle increased. He considered, on the basis of his experiments, that the extreme biological plasticity shown by *S. stercoralis* both within and without the host accounts for the existence of different strains and the varying results obtained by different workers.

Cameron (4), while describing the alternative life cycle in *Strongyloides*. states:

> "There is a single free-living generation, although under some conditions this is modified and either suppressed entirely or the females are suppressed; in other words, the young larvae give rise to only infective larvae or to males and infective larvae. The reasons for this are still far from obvious, although there is increasing evidence that they lie in the composition of the faeces."

Chang and Graham's (5) recent cytological observations of the parasitic phase of *S. papillosus* have shown that the normal number of chromosomes (six) can be seen in some ripe eggs prior to cleavage. Other eggs may show four chromosomes and an "extrusion body." In the free-living generation the diploid female pronuclei have four chromosomes. Fusion with haploid male pronuclei produces viable, triploid zygotes destined to develop into infective larvae and capable of further development into parthenogenetic, parasitic females.

Experimental Results

S. fülleborni eggs hatch outside the host and undergo predominantly indirect development under optimum conditions. *S. stercoralis* eggs hatch within the host, the rhabditiform larvae pass out in the faeces (except in hyperinfection), and development continues outside the host. *S. stercoralis* development being predominantly direct suggests the involvement of some factor limiting development of the rhabditiform larvae to the infective stage and not to the free-living adults. To determine if this is the case the following experiments were made with *S. fülleborni.*

At the optimum temperature of 25° C. *S. fülleborni* eggs hatch in from 6 to 8 hours, the first molt takes place in 10 to 12 hours, the second after 20 hours, and the third after 28 to 30 hours. Fully mature free-living adults are obtained after 48 hours' incubation.

Twenty normal cultures were set up in Petri dishes in one operation and maintained at 25° C. The cultures were baermanned consecutively at 1-hour intervals from the sixth to the 22nd hour and thousands of larvae varying from newly-hatched rhabditiform to those of the second molt were obtained in 5 to 10 cc. of clear water. At the end of each hour the larvae obtained were divided into two lots, A and B, with about 1000 in each lot. Lot A was kept in water without food; Lot B with food from the same culture: both lots were maintained at 25° C. In liquid media larval mortality is very high. The results may conveniently be considered in two groups: Group 1 with the larvae obtained before the first molt, that is, after 6 to 12 hours of incubation, and Group 2 with larvae obtained after the first molt, that is, after 12 to 22 hours of incubation.

Group 1.—In Lot A about 10 to 15% of the larvae developed into infective larvae after 48 hours; the rest all died. Lot B, to which food was added,

showed 20 to 30% of the larvae developing into the infective stage, and with them 2% of the larvae developed to immature males; the rest died. No females developed in either of these lots.

Group 2.—The individuals of Lot A all died without showing any further development. The individuals of Lot B showed some development, and immature males and females of the free-living generation were obtained. These immature males and females did not live long and died after 1 to 2 days without maturing. They are probably unable to feed properly in the liquid media and thus their normal life is restricted. It was very interesting to observe that in both these lots, no infective larvae were present.

Control experiments after 48 hours of incubation gave 100% mature, free-living females and males.

These experiments were repeated 12 times on different dates with similar results. Thus it was clear that the rhabditiform larvae developing from the eggs laid by the parasitic female, under adverse conditions develop only into infective larvae. But, if these larvae are kept under unfavorable rather than favorable conditions after the first molt has taken place, they are unable to reach the infective stage and develop into immature males and females.

On the hypothesis that rhabditiform larvae are unable to change their type of development, whether direct or indirect, after the first molt, the next step was to determine the factors responsible for the two types of development. As exogenic development is subjected to the environmental conditions of the culture media, the following factors were investigated: pH of the culture media, consistency of the culture, food available in the culture, and temperature of the culture.

pH of the Culture Media

Natural soils of different pH were used as media for the development of eggs from parasitic females. All culture media were sterilized at 250° F. at a pressure of 17 lb. to ensure that all helminth eggs were killed. The culture media used were: cow faeces (pH 8.1), Bernard loam (pH 7.05), Rideau clay (pH 5.6), Greensboro soil (pH 5.0). Charcoal powder was added to each type of media which first raised the pH slightly in each case and then kept it constant. All other conditions in each culture were similar; they were all kept at the optimum temperature of 25° C. and equal amounts of intestinal contents added. The cultures were removed from the incubator at varying intervals, baermanned, and examined.

Development in the cow faeces culture was wholly indirect. After 48 hours' incubation, mature adult males and females but no infective larvae were seen. In the Greensboro soil culture, on the other hand, direct development occurred, but in addition to the infective larvae, 10% of males but no females were obtained. As shown in Table I, the number of infective larvae increased and the number of adult females decreased as the pH of the soil was lowered. At pH 8.1 and 5.0, 10% of the individuals obtained were males: at ranges between these two pH levels, this percentage rose to 40, but the actual number

TABLE I

AVERAGE PERCENTAGE OF INDIVIDUALS OBTAINED IN DIFFERENT STERILIZED CULTURES AFTER 48 HOURS OF INCUBATION AT 25° C.

Soil culture	pH	Females	Males	Infective larvae
Cow faeces	8.1	90	10	0
Bernard loam	7.05	40	40	20
Rideau clay	5.6	10	40	50
Greensboro soil	5.0	0	10	90

of males remains about the same in all cases. The number of males which will develop from 100 eggs from a parasitic female remains constant at between 10 and 20, the remaining 80 to 90 of the eggs either do not develop or develop into free-living females or infective larvae.

In other words, there is always a constant number of eggs of the parthenogenetic female which will give rise to males; the others will give rise to females in indirect development or to infective larvae in direct development. Only the females are able to change from the indirect to the direct type of development. The males can survive to a certain extent under unfavorable conditions but beyond that the eggs destined to form males will die and will never develop to the infective stage.

Under free-living conditions the only role of the male is to fertilize the free-living female and thus increase the number of larvae which will become infective. A second generation of free-living adults does not occur. Infective larvae developed either directly or indirectly have only female primordial cells, and thus in the parasitic generation only the parthenogenetic females would develop.

Consistency of the Culture

The development of free-living stages of *Strongyloides* requires a culture of the proper consistency. If the medium is too liquid the larvae are unable to feed properly; if too solid they are unable to move and lack sufficient oxygen. The culture must be just moist and with sufficient air for normal development; otherwise, although the eggs will hatch, the development of the rhabditiform larvae is either retarded or becomes direct. To prove this, rhabditiform larvae maintained in normal cultures under optimum conditions for from 6 to 12 hours were baermanned, divided into three lots (A, B, and C) of about 1000 larvae each, and placed in Petri dishes. To Lot A only tap water was added; to Lot B tap water with food particles from the same culture; in Lot C the culture was kept in a semisolid condition.

The three lots were maintained at room temperature (23 to 25° C.) for 2 days, at the end of which the larvae were examined.

Lot A.—Eighty per cent of the larvae were dead; the balance had developed to the infective stage.

Lot B.—Seventy per cent dead, 28% infective larvae, and 2% males.

Lot C.—Fifty per cent dead, 35% infective larvae, 10% males, and 5% immature females.

In Lot C the females survived for only 2 or 3 days after the second molt but did not develop further; although a slight constriction could be seen but no vulva had formed and the gonads were reversed on both sides. The infective larvae developed normally and survived for a long time.

In experiments with solid media cultures, the mortality of the eggs was very high and those which did hatch showed no further development.

In semisolid clay media, development tends to become direct. Sterilized cow faeces was the best medium for indirect development owing to its proper consistency and exact pH.

Food Available in the Culture

Food has some effect on the development of *Strongyloides* in culture. As shown above, Greensboro soil gives direct development of *S. fülleborni.* To find whether a change in pH or the presence or absence of food is responsible for this, nutrient agar was added to Greensboro soil of pH 5.0. The control was maintained at the same pH. As will be seen from Table II, the results showed an increase in the number of males and an absence of females. In three out of 20 cultures, however, some individuals showed female morphology having reversed ovaries (but without vulva formation) on both sides of the intestine. This might be explained by assuming that the rhabditiform larvae had food for their development, allowing normal development to the second molt to take place, but further development was prevented owing to the pH of the soil so that the larvae remained nonfunctional immature females rather than developing into adult females.

TABLE II

Greensboro alone				Greensboro + agar			
No.	Females	Males	Infective larvae	No.	Females	Males	Infective larvae
1	0	12	88	1	0	15	85
2	0	8	92	2	0	12	88
3	0	11	89	3	1 abortive	19	80
4	0	10	90	4	0	22	78
5	0	14	86	5	2 abortive	25	73
6	0	10	90	6	0	18	82
7	0	12	88	7	0	30	70
8	0	13	87	8	1 abortive	24	75
9	0	12	88	9	0	15	85
10	0	13	87	10	0	22	78

All the experiments were controlled and carried out simultaneously under optimum conditions. The controls showed only indirect development to fully mature females and males.

From these experiments one can conclude that food alone is not responsible for the method of development. In the cultures of Greensboro soil to which nutrient agar had been added, there was a tendency towards indirect development. In cultures lacking the proper consistency and pH, half the larvae developed to the infective stage while of the remainder 1 to 2% changed to nonfunctional, immature females. Males do not develop to the infective stage; they remain males or die.

Temperature

All other conditions being optimum for the development of *S. fülleborni*, 25° C. will give 100% indirect development. Cultures maintained at higher or lower temperatures may show mixed development. From 20 to 30° C. development is mainly indirect, but at 15° C. (below which complete development does not take place) 5% of the eggs from parasitic females destined to form free-living females develop into infective larvae. At 35 and 37° C., although mortality is very high, about 2 per cent of the survivors become infective larvae.

Discussion

Despite the amount of work done on species of *Strongyloides*, the problem of heterogonic and homogonic development is unresolved. Studies by various workers give results that are at variance with results obtained by other workers.

The present investigation on *S. fülleborni* suggests that under normal optimum conditions there is a definite alternation of generations, a parthenogenetic one being succeeded by a single free-living sexual one. Under suboptimum conditions, however, the females of the free-living sexual generation may be suppressed or replaced by infective larvae.

Considering the parasitic generation as parthenogenetic, the different modes of development of the free-living stages cannot depend on the eggs being fertilized or not. There must be some factors in the environment which lead to the change in development from indirect to direct or vice versa.

The type of life cycle depends upon the conditions in which eggs of the parthenogenetic female hatch and pass their life as first-stage, rhabditiform larvae before the first molt. It is the stimuli affecting them then that determine which course the future, free-living development will take.

If environmental factors cause the change in the type of development, the question arises as to what are these factors and how could they explain the mixed development in one and the same culture?

The different environmental factors have already been discussed and it is clear that the type of development depends on: (*a*) pH of the culture media, (*b*) consistency of the culture, (*c*) food available in the culture, and (*d*) temperature.

The question of explaining the mixed strain in one and the same culture probably depends upon whether the hatching and the early development of the eggs of the parthenogenetic female is inside or outside of the host. *S. stercoralis* shows a predominantly direct type of development and in freshly

passed human faeces the larvae and not eggs are found. Now, because the eggs of *S. stercoralis* hatch inside the intestine of the host and remain there for some time, the first molt takes place within the host; these larvae, before the first molt, do not have favorable conditions for development into free-living adults, so they molt to the next stage, which when passed out of the intestine, can only develop to infective larvae and not to free-living adults. But, if rhabditiform larvae of *S. stercoralis* are passed outside the host body before the first molt, they will find the environment favorable and molt to free-living adults.

On the other hand, *Strongyloides* species of monkeys have a predominantly indirect development and in freshly passed faeces, fully embryonated eggs are found which hatch outside the body. The rhabditiform larvae hatched from eggs of the parthenogenetic female perhaps find suitable conditions outside the host for normal indirect development.

The finding of males in cultures with infective larvae and no females has also been explained above. As there is a definite alternation of generations in the life cycle of *Strongyloides*, the eggs of the parasitic generation would give rise to males and females of the free-living generation. That is, a definite number of males and females would develop from the eggs of the parasitic female worm. The number of males is usually smaller than that of the females among the nematodes as is also the case with *Strongyloides*. The males, however, can develop under unfavorable conditions but the female larvae unable to develop to maturity under such conditions change from rhabditiform to filariform larvae at the time of the first molt. The males, if unable to develop to maturity die, and are never able to change to infective larvae. Thus the males occur simultaneously with infective larvae in the absence of free-living females.

The cytological work on *S. papillosus* by Chang and Graham (5) has also proved that the eggs of parasitic females have chromosomal differences. Some ripe eggs show normal chromosome numbers (six), and others have four chromosomes with an "extrusion body." Thus, the eggs of parasitic females would give rise to two types of individuals in the free-living condition—females and males. Since, under adverse conditions, only the potential female larvae change to the infective stage they would naturally have all female primordial cells. In the heterogenetic or free-living condition, the eggs of the sexual female develop only after fertilization. The triploid zygote formed by the fusion of diploid female pronuclei and haploid male nuclei develop to infective larvae, having all one type of germ primordial cells. Accordingly, infective larvae developed directly or indirectly can only develop into parthenogenetic females in the parasitic generation.

Conclusions

1. There is a definite alternation of generations in the life cycle of *Strongyloides*, the parasitic generation being parthenogenetic and the free-living sexual.

2. The type of life cycle depends upon the hatching of the eggs of parasitic females, that is, inside or outside the body. The eggs hatched inside the body give rise predominantly to the direct type of generation while those hatched outside show an indirect development.

3. The stimulus causing the rhabditiform larvae hatched from the eggs of the parthenogenetic female to develop into free-living females or infective larvae must occur before the first molt; otherwise death ensues.

4. The type of development of *Strongyloides* depends upon such environment factors as pH, consistency, available food, and temperature of the culture.

5. A pH of 5.0 to 6.0 in the culture is unfavorable for indirect development and so development is predominantly direct. A higher pH, from 7.0 to 9.0, favors indirect development.

6. The consistency of the culture should be just moist and loose to permit indirect development. A change from this consistency leads from indirectness to directness.

7. The free-living adults are only developed if there is a proper supply of food. Absence of food leads to direct development.

8. Temperatures between 20 and 30° C. are favorable to indirect development, whereas lower or higher temperatures may give a mixed development.

9. The rhabditiform larvae hatched from the eggs of parasitic females give rise to a definite proportion of males and females in the free-living generation.

10. Only those larvae which would develop into free-living females are able to change into infective larvae. The males either develop to maturity or die.

11. Under comparatively unfavorable conditions the males develop normally but the female larvae change to the infective stage. Thus, males occur with the homogonic type of development as well as the heterogonic.

12. The infective larvae developed directly or indirectly have female germ primordial cells and will thus develop inside the host body only into parthenogenetic females and not to males.

13. There is only a single free-living generation. The eggs of the free-living adult females after fertilization give rise only to infective larvae.

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STUDIES IN THE MODE OF ACTION OF ROYAL JELLY IN HONEYBEE DEVELOPMENT

I. CHANGES OCCURRING IN FRESH ROYAL JELLY DETERMINED BY CARTESIAN DIVER RESPIROMETRY[1]

S. E. DIXON[2] AND R. W. SHUEL[3]

Abstract

Holter's Cartesian diver technique was used, with divers *ca.* 200 microliters volume, to study changes in royal jelly. Studies indicated that oxygen was consumed by fresh royal jelly, a phenomenon which was inhibited under an atmosphere of nitrogen. This activity diminished rapidly within a period of hours. Royal jelly held at room temperature for 6 to 8 hours showed no activity. No activity was found in fresh royal jelly which had been stored at −18° C. Cyanide did not inhibit this oxidation. The significance of the above results is discussed.

Introduction

Polymorphism in the honeybee is generally believed to be a function of nutrition. This determinism toward queens or workers seems to be set in the first 3 days of larval life (Ribbands (4, p. 246)). Worker and queen larvae are fed exclusively on a secretion of the pharyngeal glands of nursing bees, commonly referred to as royal jelly. There may be a qualitative as well as a quantitative difference in larval nutrition (2).

Using the Warburg apparatus, Melampy and Willis (3) found that oxygen consumption of 2- to 3-day-old larvae was 50% greater in queens than in workers.

We considered that a study of respiration in newly emerged larvae might provide an early, measurable criterion of polymorphism. The Cartesian diver apparatus, capable of measuring gas exchange in the range of a fraction of a microliter, should be admirably suited not only for working with limited amounts of royal jelly but also for revealing individual respiratory differences in individuals of this size.

The Cartesian diver apparatus is a constant volume respirometer in which the vessel or "diver" containing the experimental material moves in a medium of high specific gravity. The buoyancy of the diver changes as the tissue takes up or evolves gas. The pressure required to restore the original gas volume in the diver is read from a manometer.

Preliminary respiratory measurements showed that in royal jelly itself there was gas uptake. We decided therefore to investigate this phenomenon before proceeding with respiratory studies. The results of this investigation are reported in this paper.

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Joint contribution from the Department of Entomology and Zoology and the Department of Apiculture, Ontario Agricultural College, Guelph, Ontario.
[2]Department of Entomology and Zoology.
[3]Department of Apiculture.

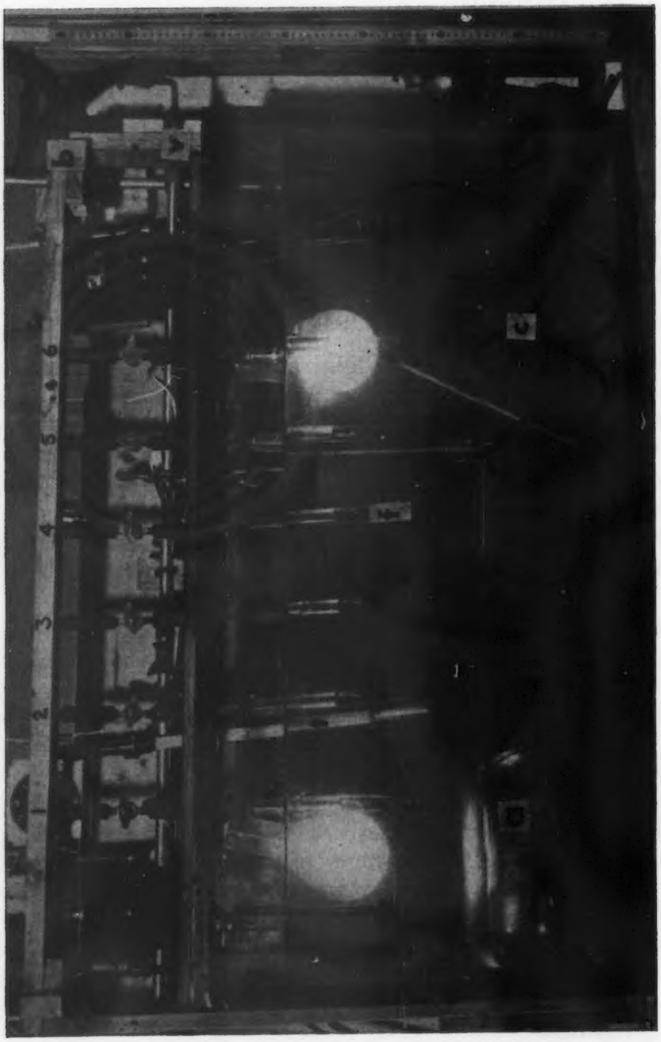


FIG. 1. Cartesian diver apparatus: *a*, air bottle; *b*, three-way stopcock to air bottle; *c*, cooling coil; *f*, flotation vessel containing diver; *t*, thermoregulator; *v*, three-way stopcock to vessels.

Materials, Methods, and Apparatus

The apparatus as developed by Holter (1) was used with minor modifications (Fig. 1). Connections between the air manifold and the flotation vessels were kept as short as possible.

Experiments were conducted at a temperature simulating that of the hive, 35° C. The bath temperature was maintained by a thermoregulator and electronic relay at 35° plus or minus 0.01° C., the room temperature at 33° plus or minus 1° C. A trickle of cold water was allowed to run through the copper cooling coil in the bath. The frequency of manometer readings was determined by the activity of the jelly. Divers, made of 9 mm. O.D. pyrex glass tubing, were of the bulb type, with bulbs of *ca.* 6.5 mm. diameter, stems 2 cm. in length, and volumes between 150 and 200 μl. The neck bores were 1.9 to 2 mm. diameter. Diver tails were made from solid pyrex cane. Seals were about 5 μl. in volume. Seals less than 4.5 μl. would not spread across the neck of divers of the size used. Loose-fitting solid glass stoppers were used in the oil seal, or, if the charge was to be changed during the experiment, in the mouth seal. With 5 μl. seals (total seal volume 15 μl.), the ideal weight/volume ratio for a diver was found to be 3.05 to 3.07. This figure was determined empirically for a test diver and tails of all divers were weighted to give this ratio. Diver volumes were determined by weighing the divers (containing the glass stopper) before and after they were filled with water. Divers can be made to fall within the equilibrium pressure range by adjusting for the volume and weight of biological material under test (charge).

Charging Divers

The apparatus for charging divers is shown in Fig. 2. It consisted of a manifold containing a pipette for each of the three seals and one for the royal jelly, mounted above a manipulator. The manipulator consisted of a microscope barrel mounted on a sliding microtome. Into the microscope barrel a microtome stud holder was fitted. This combination permitted movement in all planes. Divers were inserted into a silicone plug fixed on top of the microtome stud holder. Pipettes were fixed in position in the manifold and the divers were moved up to them. Filling and emptying of pipettes was accomplished by sucking or blowing through a rubber tube attached to the manifold. The pipettes for the seals were of the self-filling type, made from thick-walled pyrex tubing of 1 mm. bore. Ball tips were put on the pipettes. The size of the ball tip is critical, about one and one-half times the diameter of the pipette. If the ball tip is smaller, the ball of fluid is too small to make contact with the sides of the diver neck; if larger, the sides of the neck are smeared.

Royal Jelly

The royal jelly was collected by the method of Smith (5) in which artificial queen cells are grafted into a frame and introduced into the hive. Royal jelly of a known age can be removed from the cells by suction.

FIG. 2. Apparatus for charging divers: *d*, diver; *h*, microtome stud holder; *m*, pipette manifold; *p*, pipette.

For introducing the royal jelly into the Cartesian diver, an extra pipette with a bore of about 1 mm. was mounted in a manifold. Five microliter samples were used. At 35° C., newly secreted jelly is quite fluid and fairly easily pipetted.

Charging Divers in an Atmosphere of Nitrogen

For experiments conducted in an atmosphere of nitrogen a cylinder of bone-dry nitrogen was connected through a pyrogallol train to the pipette manifold and also to the sidearm of a glass nitrogen mantle which fitted over the silicone plug in which the Cartesian diver rested for filling (Fig. 3).

The entire manifold was flushed with nitrogen for 10 minutes before the divers were charged. The royal jelly was expelled into the diver under pressure of nitrogen and the diver was then thoroughly flushed with nitrogen before the lower neck seal was inserted. A positive pressure of nitrogen was maintained in the mantle surrounding the diver.

The efficiency of this flushing procedure was demonstrated by dissolving a crystal of pyrogallol in the lower neck seal (sodium hydroxide). No more than a very slight amber color developed in the hydroxide at any time.

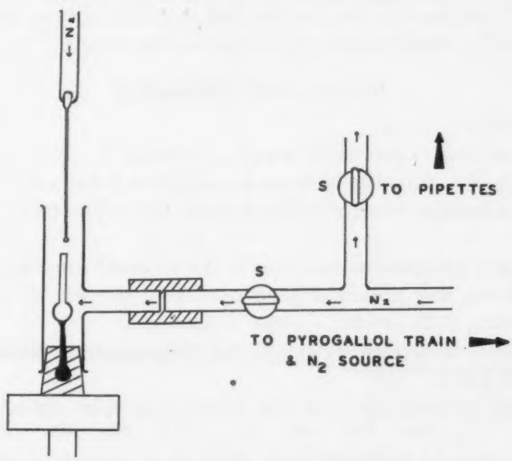


FIG. 3. Apparatus for charging divers under an atmosphere of nitrogen. Small arrows indicate direction of nitrogen flow.

Measurement of Gas Exchange

A half-hour equilibrium period was used before the initial reading was taken. A control diver was used in all experiments as a check on any pressure deviations in the system. The divers were not submerged between readings, but were exposed to one atmosphere pressure when individual stopcocks were closed. Although this was a departure from the procedure of Holter (1) it was found that where an average pressure of 20 cm. was required to submerge

a diver the accumulated pressure required to submerge six divers separately sometimes exceeded the range of the manometer (about 100 cm.). The constancy of the control diver readings suggested that this procedure was valid.

Errors in Measurement

The possible error of a manometer reading was plus or minus 0.05 cm. The standard deviation of 67 readings of control divers was plus or minus 0.13 cm. This value could have been reduced by ensuring that glass stoppers fitted very loosely. A tight-fitting stopper, a dirty diver, or dirt in the oil seal will inhibit ready response of the oil seal to a pressure change in the system.

Seals

In many divers with neck diameters of the order of 2 mm. it is advisable to use a wax ring at the base of the neck to prevent the hydroxide seal from creeping down the side of the bulb to the charge. Where carbon dioxide is not evolved, a sodium chloride solution may be substituted for hydroxide in the lower neck seal. In the present experiments no carbon dioxide was evolved, and a sodium chloride solution was used for the lower neck seal. The vapor pressure of the royal jelly charge (6) is very close to that of the seal (2 M NaCl) or 2 N NaOH. In nitrogen experiments, where pyrogallol in NaOH in the lower neck seal was used to test for oxygen-free atmosphere, creeping of the seal was avoided by placing the seal well up in the neck.

Results and Discussion

Royal Jelly in Air

The results of two experiments on gas exchange in royal jelly in air are presented in Fig. 4. Royal jelly from a number of 1-day-old queen cells was pooled to give a homogeneous sample on each day. The following inferences seem justified:

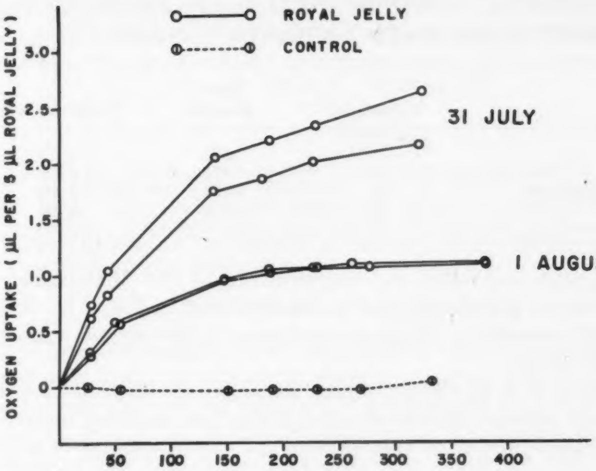
1. There was a progressive decrease in the internal pressure of the atmosphere in the diver, due presumably to uptake of oxygen by the royal jelly. After 5 or 6 hours, little further change occurred.
2. There was close agreement between duplicate subsamples from the same sample of royal jelly.
3. There was considerable variation between samples collected on July 31 and August 1.

Further experiments indicated that these data were typical of royal jelly from 1-day cells.

Part of the sample of royal jelly collected on August 1 was held at room temperature for 24 hours, then tested for activity. None was found. Similarly, a sample of 1-day jelly stored for 4 weeks at minus 18° C. was inactive. These results would indicate that some constituent present in fresh royal jelly was being rapidly oxidized.

Royal Jelly in Nitrogen

The data in the graph in Fig. 5 shows that no activity was present in fresh royal jelly under an atmosphere of nitrogen. This lack of activity in nitrogen,



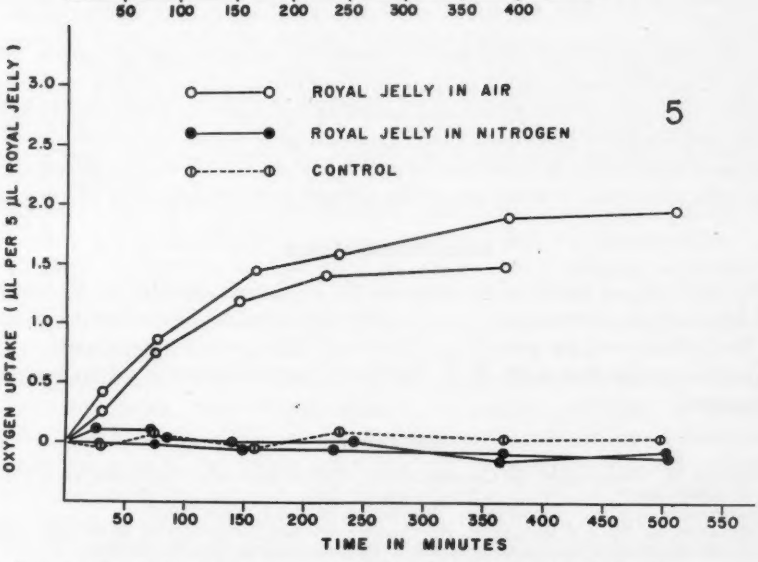


FIG. 4. Graph showing oxygen uptake of two samples of royal jelly in air.
FIG. 5. Graph showing effect of nitrogen atmosphere on gas uptake by royal jelly.

compared with the activity in air of the control subsamples taken from the same sample of royal jelly, demonstrates that an oxidative process was taking place.

The ephemeral activity of freshly secreted royal jelly suggested that this oxidation might be enzymatically controlled. If the activity were due to the presence of an oxidase system in fresh jelly, it should be inhibited by cyanide. Data from an experiment in which gas exchange was measured in royal jelly

TABLE I

RESPIRATION OF ROYAL JELLY IN THE PRESENCE OF CYANIDE

Treatment	Subsample	Time, minutes	O_2 uptake (μl).
Royal jelly control	1	170	0.69
	2	172	0.61
Royal jelly + NaCN (10^{-2} *M*)	1	174	0.70
	2	179	0.69

to which sodium cyanide was added at a concentration of 10^{-2} *M* (buffered to pH 4.0, the approximate pH of royal jelly) are presented in Table I. Since oxidation proceeded normally in the presence of cyanide, the oxidative process is probably non-enzymatic.

Physical phenomena such as vapor pressure, solubility, or sorption of gases in royal jelly cannot account for the striking differences manifest under an atmosphere of nitrogen—nor could they explain the loss of activity in stored royal jelly.

Under hive conditions royal jelly is constantly secreted into the queen cell for larval nourishment, and it is these later additions which exhibit oxidative activity. Because of the ephemeral nature of the activity, it is unlikely that it is a causal factor of polymorphism. Queens can be reared on stored jelly (6). Studies of respiration in honeybee larvae, however, should be conducted with royal jelly more than 6 hours old, if the activity is not to influence the results.

Acknowledgments

The authors are indebted to Professor G. F. Townsend, Dr. M. V. Smith, and Mr. A. Adie of the Apiculture Department, Ontario Agricultural College, for their advice and the provision of the royal jelly used in experiments.

Thanks are also due to Dr. R. L. Patton of Cornell University, who read the typescript.

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CORYNOSOMA MAGDALENI SP. NOV. (ACANTHOCEPHALA), A PARASITE OF THE GRAY SEAL IN EASTERN CANADA[1]

PAUL L. MONTREUIL[2]

Abstract

Corynosoma magdaleni sp. nov. (Acanthocephala) is described from the gray seal, *Halichoerus grypus* (Fabricius), and the Atlantic harbor seal, *Phoca vitulina concolor* (DeKay), the latter as an infrequent accidental host in Eastern Canada. Juveniles were found in halibut, *Hippoglossus hippoglossus* (Linn.) and shorthorn sculpin, *Myoxocephalus scorpius* (Linn.) in the Magdalen Islands region of the Gulf of St. Lawrence.

Materials and Methods

The present morphological descriptions and discussion of variation are based on a study of 405 adult specimens collected from five gray seals and one harbor seal, and on two juveniles (one each from halibut, *Hippoglossus hippoglossus* (Linne.), and shorthorn sculpin, *Myoxocephalus scorpius* (Linn.)).

Most of the host digestive tracts were examined fresh, or after storage frozen for varying lengths of time. All morphological studies were made on material collected from fresh intestines. To relax the worms, they were placed in fresh water, and refrigerated until the proboscis was well everted. They were then fixed in warm A.F.A. (alcohol – formaldehyde – acetic acid), or in Gilson's fluid, subsequently washed free of unwanted reagents, and stored in 70% alcohol.

Ehrlich's haematoxylin, combination haematoxylin of Van Cleave (7), chlorazol black, Grenacher's borax carmine, Mayer's paracarmine, and Schneider's acetocarmine were used as stains. Paracarmine was used to stain most of the material as it gave the most satisfactory results. In a few instances, when it was desired to bring the spines out in sharper contrast, the indigo carmine method followed by Moore (4) was used.

Stained specimens were dehydrated in alcohol, cleared by passage through successively more concentrated solutions of methyl salicylate in absolute alcohol, and finally stored in pure methyl salicylate until studies on them were terminated, or until the specimens were ready to be mounted. All measurements and most photographs and camera lucida drawings were made from unmounted, cleared specimens.

While working on *Corynosoma* spp. a technique was evolved for making consistently good whole mounts in balsam without the necessity of piercing the cuticle. This method calls for a graded series of three mixtures of methyl benzoate – terpineol and balsam. The worms are passed from methyl salicylate to the first of these, left for a few hours, then transferred to the weak balsam mixture and left until they sink to the bottom of the container. They can then

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[2] Director, Marine Biological Laboratory, Grindstone, Magdalen Islands, Que.

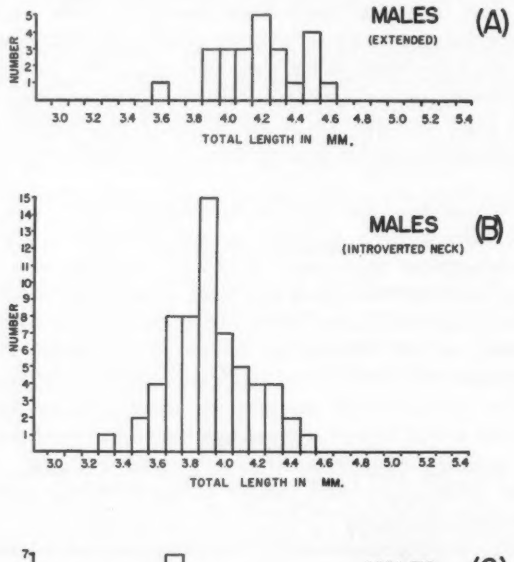
be brought to a strong balsam mixture, allowed to sink again, and finally mounted in pure balsam. Specimens prepared in this manner show no signs of the distortion which is often so hard to avoid in the Acanthocephala, and mounts prepared 3 years ago are as perfect now as when first made.

Corynosoma magdaleni sp. nov.

Palaeacanthocephala (Meyer, 1931) Van Cleave, 1948 (2, 5); Polymorphidae Meyer, 1931; Polymorphinae Meyer, 1931; *Corynosoma* Lühe, 1904 (1).

In the ensuing description, modal values are given for the measurements. Variation is discussed following the description.

This species is the slenderest of any member of the genus *Corynosoma* described to date from North American mammals. Mature females are usually slightly longer than males, most being around 4.5 mm. (fully extended), while most of the males have a total length close to 4.2 mm. (Figs. 1–3). The



FIGS. 1–3. Distribution of lengths, demonstrating the effect of introversion of neck and tail on the total length in males and females. Total length in mm.; class interval equals 0.1 mm.

FIG. 1. Distribution of lengths of males: A, fully extended; B, neck only retracted; C, neck and tail introverted.

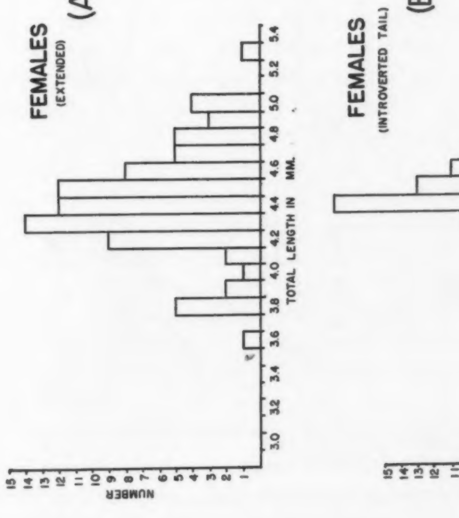


FIG. 2. Distribution of lengths of females: A, fully extended: B, tail introverted.

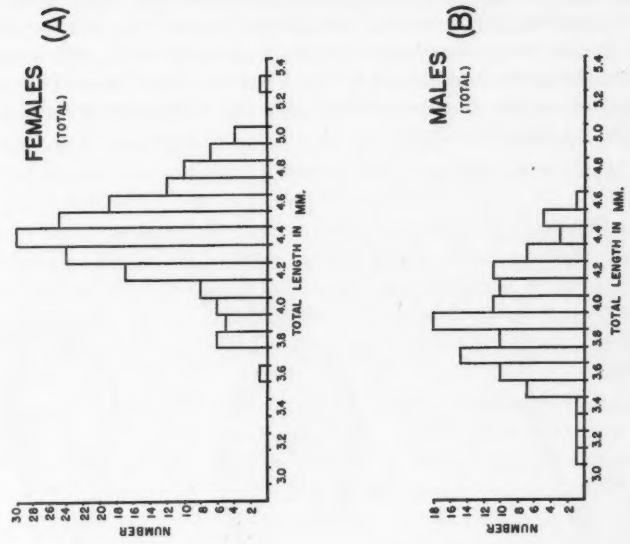
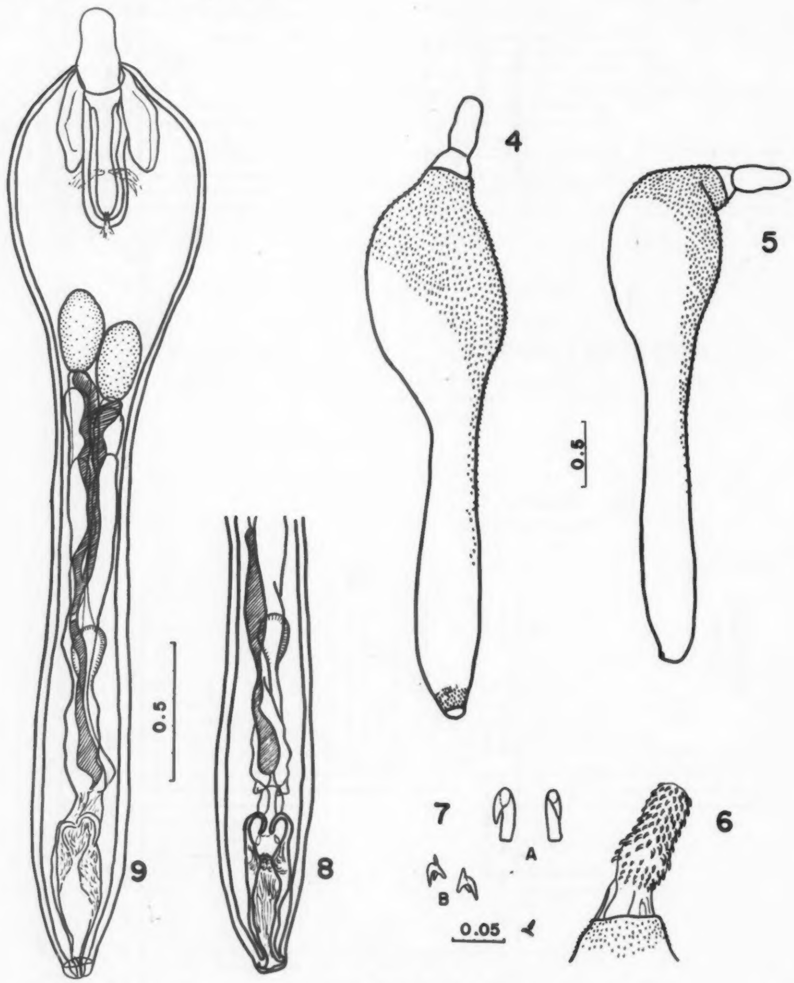


FIG. 3. Distribution of lengths of total collection: A, females; B, males.

fore-trunk is not very sharply set off from the hind-trunk, there being a fairly gradual attenuation of the former posteriorly to meet the hind-trunk (Figs. 4, 10). The fore-trunk usually is 1.3 mm. to 1.6 mm. long, with a maximum dorsoventral diameter in males of 0.8 to 1.0 mm. The female fore-trunk is slightly thicker, on the average, with a diameter of between 0.9 and 1.1 mm., occasionally reaching 1.2 mm.



FIGS. 4–9. The 0.5 mm. scale between Figs. 8 and 9 applies also to Fig. 6.
FIG. 4. Body form and trunk spination of mature male.
FIG. 5. Body form and trunk spination of mature female.
FIG. 6. Proboscis and praesoma.
FIG. 7. Hooks and hook roots: A, anterior series; B, basal series.
FIG. 9. Ventral view of mature male showing internal organization; same specimen as Fig. 8, which shows lateral view.

The hind-trunk is proportionately long and slender, slightly under twice the length of the forebody, and slightly thinner at the mid-point along the length of the worm than it is farther posteriorly (Figs. 4, 5, 9). The minimum diameter varies from 0.35 to 0.37 mm. and the maximum diameter of the hind-trunk close to the posterior extremity is usually about 0.43 to 0.45 mm.

The fore-trunk extends anteriorly and somewhat ventrally to form a base from which the neck arises. This base is about as long as its distal diameter where it joins the neck (Figs. 4, 6, 10).

The neck has the form of a short, truncated cone of obtuse angle. The diameter of its base is about 0.30 to 0.33 mm. The length of the neck is between 0.14 and 0.17 mm. and rarely reaches 0.19 mm. The diameter of the distal extremity of the neck (base of the proboscis) is about 0.14 to 0.16 mm. in the male, and often slightly in excess of this in females, where it can attain 0.18 mm.

The proboscis is somewhat vase-shaped, with a swelling in its basal third, as is typical of most species of *Corynosoma*. The length is usually between 0.44 and 0.50 mm., and tends to be slightly greater in females than in males. The maximum diameter of the proboscis, at the level of the swelling in the basal third varies from 0.19 to 0.22 mm., in the male, while in the female it is usually between 0.23 and 0.25 mm. The sides of the anterior half of the proboscis are nearly parallel, with a diameter at this point of 0.16 to 0.18 mm.; this measurement in females often slightly exceeds that in males (Fig. 6).

The proboscis armature (Fig. 6) consists typically of 20 longitudinal rows of hooks, but specimens are found with 17 to 23 rows. The range of 19 to 21 rows of hooks includes about 90% of the specimens examined (Figs. 16, 17). There are 10 to 11 hooks per row, rarely 9. They are differentiated into an anterior series of 6 or 7, bearing strong, recurved roots which are longer than the thorns (Fig. 7A). The basal 4 or 5 (rarely 3) hooks are much smaller, closely crowded, and separated from the anterior hooks by a wide interval. Their roots are in the form of an inverted "Y", the leg of which is directed anteriorly, and which is up to twice as long as the diameter of the thorn. The two posteriorly directed branches of the root are quite distinct, and their length is equal to, or slightly greater than the diameter of the thorn (Fig. 7B).

Although the roots of the large hooks are often noted in descriptions of the Polymorphinae, those of the small basal proboscis hooks are usually ignored, or said to be lacking (particularly in descriptions of *Corynosoma* and *Polymorphus*). The presence of roots on these small hooks has been ascertained by the author in several species, and it is believed that these can be of considerable assistance in the classification of the group. This subject will be discussed more fully at a later date.

The largest hooks, occurring at the level of maximum diameter of the proboscis, usually have a length of 0.050 to 0.060 mm., and the width at the base is 0.019 to 0.023 mm. Measurements of the anterior hooks of the basal series gave the following values: length 0.023 to 0.036 mm., width 0.008 to 0.014 mm.

Trunk spines cover the extension of the forebody which forms the base supporting the neck. Dorsally, they extend only a short distance beyond this point along the forebody. Ventrally, they cover the forebody, and extend posteriorly in reduced numbers, along the ventral surface of the hind-body to terminate a short distance behind its narrowest portion. The pattern of trunk spination is similar in both sexes (Figs. 4, 5). Trunk spines are usually sigmoidal, not prominent, but are clearly discernible: their length lies usually between 0.025 and 0.030 mm.

Numerous spines surround the genital opening of the male (Fig. 4, 11, 12). These cover the entire circumference of the tip of the hind-body, extending anteriorly to a point approximately mid-way between it and the level of maximum diameter of the hind-body. The length of the genital spines in the male is usually between 0.020 and 0.025 mm. The posterior extremity of the hind-trunk is often invaginated, forming a "genital vestibule" into which the spinose tip is withdrawn (Fig. 13).

Small numbers of genital spines (2 to 10) are present in less than half of the females, and show the same range of variation of length as for males.

The proboscis receptacle is a closed, double-walled, muscular sac, typical of other members of this genus (Fig. 10). Its length in males usually lies between 0.60 and 0.65 mm.; in females, it is somewhat longer, usually between 0.65 and 0.70 mm. The brain, a small ganglion, lies in the proboscis receptacle, on the ventral surface about mid-way along its length, or slightly closer to the posterior extremity. The lateral posterior nerves pierce the wall of the proboscis receptable ventrally, at the level of the ganglion, on either side of the mid-line of the receptacle, separated by an interval of 0.050 to 0.055 mm. (Fig. 9). The retinacula are directed laterally and posteriorly, and join the inner wall of the forebody. The receptacle retractors emerge from the posterior extremity of the proboscis receptacle in two places (Fig. 10). Of these, one is slightly subterminal and dorsal, and extends posteriorly to terminate on the dorsal surface of the hind-trunk wall, after branching

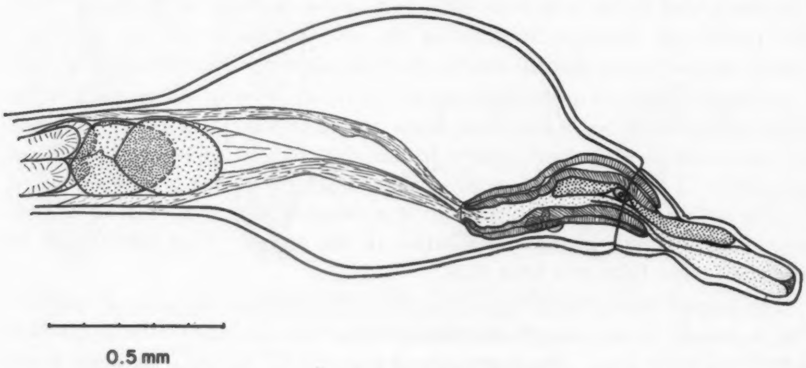


FIG. 10. Morphological details of the proboscis and proboscis receptacle.

into two strands mid-way between the insertion and origin. The ventral retractor is terminal on the proboscis receptacle, and is associated with the ligament sac; it terminates on the ventral wall of the hind-trunk, farther back than the dorsal retractor. Observation of this fact is rendered difficult in whole mounts by the ligament sac which seems to surround it anteriorly, and which encloses the reproductive glands in the male as well (genital sheath).

A structure first noticed in the proboscis of this species, and subsequently found in several others does not seem to have been discussed or described by previous workers. A large mass of tissue is situated dorsally on the inner surface of the proboscis and proboscis receptacle (Fig. 10). It is flattened dorsoventrally, evenly rounded anteriorly, and extends to a level just anterior to the circle of largest hooks on the proboscis, giving a shield-like appearance from a dorsal aspect. Its width, anteriorly, is almost equal to that of the base of the proboscis. It extends posteriorly into the receptacle where it terminates at a point halfway along its length. Its thickness in the bulbous part of the proboscis is approximately 0.090 mm. A "ganglion" occurs on the mid-line of the dorsal surface, at the level of the neck base in fully extended specimens (Fig. 10 shows an optical section through the "ganglion").

The tissue of this mass has a homogeneous, slightly granular appearance. The portion within the proboscis seems distinctly separated from other tissues. In the proboscis receptacle, it is in close contact with the inner surface of the inner muscular layer.

In the original description of *Polymorphus capellae*, Yamaguti (8) mentioned the presence inside the proboscis of two saccular vesicles of unknown function for which he proposed the name "proboscis vesicle." These are not illustrated and their description is far from adequate. Whether the "proboscis vesicle" of Yamaguti and the structure described above are the same remains to be determined.

The lemnisci in this species are broad, leaf-shaped, often with recurved edges, and almost invariably shorter than the proboscis receptacle. In some specimens in which the neck is retracted, the lemnisci assume a funnel-shaped appearance.

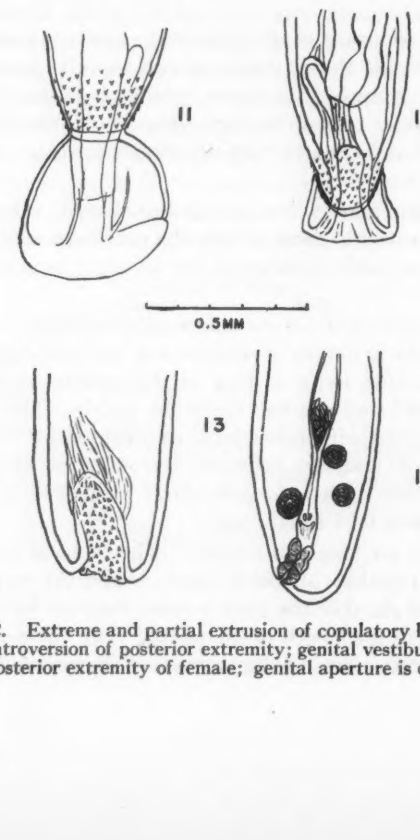
The reproductive system of *Corynosoma magdaleni* differs from other species of the same genus only in details of relative size and proportions. The testes are ellipsoidal, sometimes twice as long as they are wide, but the length is usually only about one and one-half times the width. The testes, of course, are slightly elastic and their proportions are affected by their position. In the narrow hind-trunk, they are long and narrow, whereas in the fore-trunk, they are more rounded. Their lengths range from 0.32 to 0.43 mm., with most common values of 0.24 to 0.27 mm.

The cement glands are long and narrow. The bulbs of the anterior pair of each triad are close together, almost in contact with the corresponding testis. The bulb of the third gland of the triad is some distance behind the testis, the interval being equal to the length of the testis. The ducts of each triad unite at a considerable distance behind the junction of the sperm ducts. The latter

unite 0.8 to 0.9 mm. behind the testes. Saefftigen's pouch is usually about 0.8 mm. long and reaches anterior from the apparatus of the bursa to within 0.2 to 0.3 mm. of the junction of the sperm ducts (Figs. 8, 9).

The position, shape, and size of the testes, and their relation to other portions of the body have often been ascribed considerable importance as a diagnostic feature among other species of this genus. Several specimens of *C. magdaleni* showed that this character is extremely variable, and depends on the condition of the specimen at the time of fixation. As the genital apparatus is continuous with the eversible copulatory bursa at one end, and is attached (by the sheath and ligament sac) to the proboscis receptacle at the other, it follows that any variation in the state of inversion or extrusion of the bursa, tail, or praesoma will cause corresponding changes in gonad position and shape.

Ovarian balls in gravid females range from 0.07 to 0.11 mm. in diameter; they are spherical to slightly ellipsoidal. The uterus is as in other species, with a thin-walled portion which is usually distended with shelled embryos posterior to the uterine bell. Posteriorly, the walls of the uterus thicken to form a thin tube of 0.35 to 0.45 mm. in length and 0.025 to 0.060 mm. in diameter. The uterus thickens 0.050 to 0.060 mm. anterior to the uterine sphincter at the point of formation of the vaginal funnel. The uterine sphincter is constricted in the middle giving it a squat, hourglass shape, and



FIGS. 11–12. Extreme and partial extrusion of copulatory bursa in the male.
FIG. 13 Introversion of posterior extremity; genital vestibules.
FIG. 14. Posterior extremity of female; genital aperture is dorsal.

PLATE I

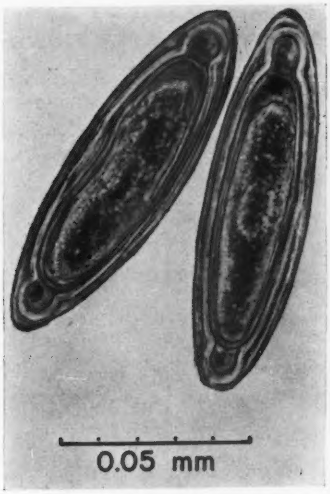


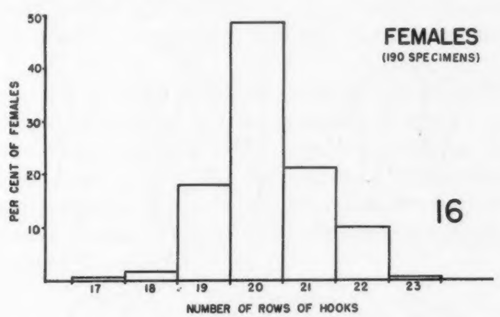
FIG. 15. Eggs.

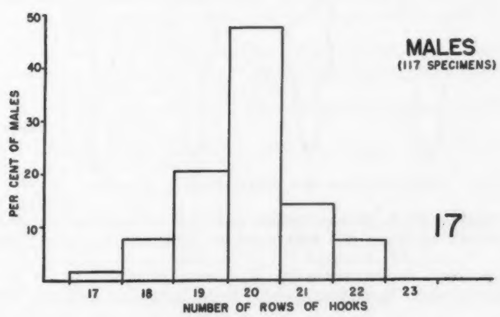
measures from 0.085 to 0.100 mm. in length. The diameter is generally equal to its length. The vagina has a length of 0.045 to 0.070 mm., and a diameter of 0.055 to 0.060 mm. The female genital pore is subterminal and dorsal, being situated near the tip of the hind-body, on the dorsal surface at the beginning of the terminal curve of the body wall (Fig. 14).

The shelled embryos of this species are 0.075 to 0.090 mm. in length, and 0.025 mm. in width. The second "shell" forms a prolongation at both poles (Fig. 15). Yamaguti (9) discussed the shrinkage of the shelled embryos of *C. phalacrocoracis* which occurs when they are mounted in balsam. The same phenomenon was observed in the case of *C. magdaleni*: Fig. 15 illustrates unmounted eggs.

Detailed measurements were made on specimens of each sex having both extreme and modal number of rows of hooks on the proboscis. For this species at least the number of longitudinal rows of hooks on the proboscis is a variable character which is independent of other characters.

Hosts.—First intermediate host unknown. Second intermediate hosts, halibut and shorthorn sculpin. Definitive host, gray seal and harbor seal. Harp seal, which occurs seasonally in the Magdalen Islands region, does not seem to carry this species.



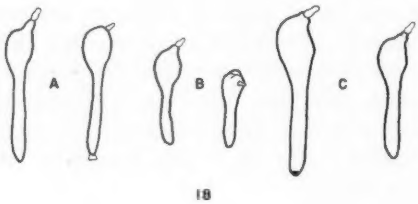


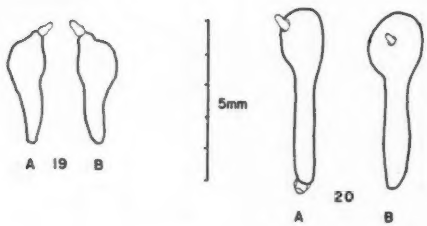
FIGS. 16 AND 17. Variation in number of longitudinal rows of proboscis hooks in 190 female and in 117 male *Corynosoma magdaleni*.

TABLE I

SEX RATIO OF *Corynosoma magdaleni* IN SEALS

Host	Male	Female
Gray seal No. 298	1	5
Gray seal No. 299	129	195
Gray seal No. 300	2	12
Gray seal No. 401	2	5
Gray seal No. 402	6	46
Harbor seal No. 243	1	1
Total	141	264





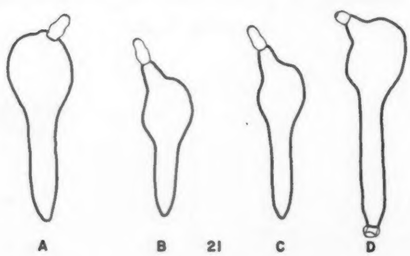


FIG. 18. *C. magdaleni*: A, mature males, one with extroverted copulatory bursa; B, juvenile males recovered from fish intermediate hosts; C, mature females, one with copulatory cap.
FIG. 19. *C. semerme*: A, mature male; B, mature female.
FIG. 20. *C. strumosum*: A, mature male with extroverted copulatory bursa: B, mature female.
FIG. 21. *C. hadweni*: A, mature female; B, juvenile female from fish intermediate host; C, juvenile male from fish intermediate host; D, mature male with extroverted copulatory bursa.

Type material.—Type specimens P.M. 299-2 (one male and one female) from gray seal *Halichoerus grypus*, Deadman Island, Gulf of St. Lawrence, Quebec, January 26, 1954, to be deposited in the U.S. National Museum. Paratypes representing both sexes, from the same host, to be deposited in the Institute of Parasitology, Macdonald College, Que.

Habitat.—The habitat of *Corynosoma magdaleni* is strictly localized in the intestine of the host. In all cases, the total infection was found within the last 7 feet of small intestine, or, exceptionally, in the colon. Individuals were found in the colon (one female and two males in host 299: two females and one male in host 402) only in the case of the two heaviest infections, and were apparently old, almost spent specimens.

Sex ratio.—The sexes are distributed as shown in Table I. The males in the aggregate are outnumbered by the females in a ratio approaching 2:1. Because of its strict localization in the intestine, it was not possible to determine whether the sex ratio is correlated with the habitat.

Incidence and mixed infections.—*C. magdaleni* was found in five gray seals out of a total of six examined, and in one of two harbor seals, all of which were obtained between 1953 and February, 1955. None of the nine harp seals examined in the spring of 1954 carried this species. All hosts which carried *C. magdaleni* were also infected with *C. hadweni* Van Cleave, 1953 (6), and two of the gray seals carried a third species, *C. semerme* (Forssell, 1904). The heaviest infection with *C. magdaleni* was in a nursing female which harbored 324.

Comparisons.—*Corynosoma magdaleni* most closely resembles *C. strumosum* and *C. hadweni* in body form but is considerably smaller than either in all its proportions. The proboscis bears a greater number of rows of hooks, which are smaller than in the other two species. The trunk spination differs from that of other species in that it extends farther posteriorly on the ventral surface of the hind-trunk. Figs. 18 to 21 illustrate the body outlines of these three species and of *C. semerme.*

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PARASITES OF NYMPHAL AND ADULT GRASSHOPPERS (ORTHOPTERA: ACRIDIDAE) IN WESTERN CANADA[1]

R. W. SMITH[2]

Abstract

The taxonomy, distinctive features, life histories, seasonal occurrence, incidence of parasitism in different hosts and at different times, and the economic significance of 23 species of primary parasites of grasshoppers are described. Three species of hyperparasites are similarly discussed. The information is based on field collections made in Western Canada in 1938 to 1953 and on laboratory studies at Belleville in 1939 to 1955.

Introduction

The early-stage larvae of parasites found in nymphal and adult grasshoppers in Canada were described by Smith and Finlayson (63) in 1950, but without reference to the relative importance of the species. The following includes a brief review of the taxonomic history and occurrence of each species as reported in the literature, and an account of its life history and habits, seasonal occurrence, distribution, and host associations.

The information was obtained by officers of the Entomology Division in an investigation of parasites of grasshoppers in Canada. Approximately 20 localities in the Prairie Provinces were selected as typical habitats of pest species of special interest by, or under the direction of, the late Mr. H. W. Moore, Entomology Laboratory, Saskatoon, Saskatchewan. In British Columbia some 10 localities that represented typical variety of range conditions were selected by Mr. E. R. Buckell, formerly of the Entomology Laboratory, Kamloops, and by Prof. G. J. Spencer, University of British Columbia. Most of the field collections of grasshoppers were made by officers in field crop insect work, but some were made by officers in biological control work.

Series of samples were taken annually at approximately semimonthly intervals from mid-June to late August or mid-September. Each sample was taken with a net from a mixed population of grasshoppers, placed in 70% methyl alcohol, and subsequently transferred to 70% ethyl alcohol before it was sorted to species and instar. The sorting and the recording of host data were done initially at Brandon, Manitoba, and later at Saskatoon. The sorted samples were sent to Belleville to be examined for parasites. Each grasshopper was examined for parasites as follows: it was removed from the preservative, pinned on a firm rubber block with its ventral surface uppermost, cut open along the mid-ventral surface with fine dissecting scissors, and its head and body cavities examined for parasites with a binocular microscope (×20). Identification of larvae at times required magnifications of ×400.

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Contribution No. 3591, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.
[2]Entomology Laboratory, Belleville, Ontario.

Detailed records were made of the species and stage of the host and of parasites it contained. It was also noted whether each parasite was living or dead when the host was collected; melanized parasites were considered as dead.

In addition to the grasshoppers preserved for dissection, collections of living grasshoppers from Western Canada were sent to Belleville for rearing. These provided stocks of living parasites for propagation, for studies of their life histories and habits, and for identification of larvae to species. For shipment to Belleville the grasshoppers were placed in 25-lb. paper bags with small amounts of grass or other vegetation to provide food. These bags were placed in cartons and forwarded by rail express. Collections from British Columbia were usually sent by air express. Living grasshoppers that reached Belleville as many as 5 days after collection in the field usually arrived in good condition. At Belleville, the grasshoppers were kept in cylindrical cages for emergence of parasite larvae. The cages were 5 in. in diameter and 10 in. in height, and were made of 16-mesh wire screening for the smaller nymphs, and of 12-mesh screening for the larger nymphs and adults. Blades of wheat or orchard grass and leaves of lettuce or dandelion (*Taraxacum officinale* Weber) were provided as food. The cages were set in funnels in racks, and the emerged parasite larvae dropped through the floor of the cage into containers below. Emerged larvae were transferred each day to screen-stoppered vials (2.0 in. × 0.5 in.) or to flowerpot-saucer containers for pupation and emergence of adults. Larvae that failed to pupate were placed in cold storage at 0° or 8° C. for 30 or more days to satisfy the diapause requirements. Puparia of tachinids or muscids that failed to produce adults within a reasonable length of time were given a similar cold treatment.

Adult parasites captured in the field were a source of larvae for laboratory propagation and of information on distribution and seasonal occurrence. No surveys were made for the express purpose of determining distributions of the parasites and, apart from specimens in the collections of Western laboratories, the records of distributions are limited largely to the localities from which the annual collections of grasshoppers were made.

Buckell and Spencer (15) in 1945, in their preliminary list of the flesh flies of British Columbia, recorded the following parasites of *Melanoplus mexicanus* (Sauss.), not all of which were encountered in the present investigation: *Acridiophaga aculeata* (Ald.), *A. aculeata* var. *gavia* (Ald.), *A. aculeata* var. *taediosa* (Ald.), *Blaesoxiphotheca coloradensis* (Ald.), *Helicobia rapax* (Wlk.) [*Sarcophaga rapax* Wlk.], *Kellymyia kellyi* (Ald.) [*Sarcophaga kellyi* Ald.], *Opsophyto opifera* (Coq.) [*Sarcophaga opifera* Coq.], *Protodexia hunteri* (Hough) [*Sarcophaga hunteri* Hough], *Sarcophaga exuberans* Pand., *S. falciformis* Ald., *S. harpax* Pand., *S. reversa* Ald., *S. sarracenioides* Ald., *Sarcotachinella sinuata* (Mg.) [*Sarcophaga sinuata* Mg.].

Some of these may be represented in the material collected in the present investigation by larval stages not yet associated with their adults. With few exceptions, such as an occasional unidentified female obtained by rearing

or an occasional unrecognized larva found by dissection, all species encountered in this study are discussed below. They are treated alphabetically within their family groups.

Sarcophagidae

Except where noted the following sarcophagids all have a double-sac incubating uterus and deposit living first instar larvae on the host. The larvae gain entry with their mouth hooks through the more lightly sclerotized parts of the integument such as those around the bases of the legs and wings, or in the region of the occiput. The species that deposit larvae may place them on various parts of the body and, according to the observations of ourselves and others, they may place them on hosts in the air or at rest.

There are three larval stages, all free-living within the body of the host. No evidence of attachment of larvae to the tracheae or air sacs of the host was observed although this probability has been suggested by de Crouzel (29). The mature larvae leave the host and enter the soil for pupation. Winter is passed in the larval stage in the soil.

ACRIDIOPHAGA ACULEATA (Ald.)

Aldrich (2) described this species in 1916 as *Sarcophaga aculeata* and recognized two varieties, *gavia* and *taediosa*. In 1917 Townsend (73) established the genus *Acridiophaga* with *S. aculeata* as type species. The holotype male and allotype female are from Wellington, Kansas. The holotype male of *gavia* is from Koehler, New Mexico, and a specimen probably of this variety was from New Jersey. The holotype male and allotype female of *taediosa* are from Brookings, South Dakota, and other males were from New Mexico, South Dakota, and Wyoming.

Specimens examined by Aldrich included males reared from *Melanoplus spretus* (Walsh) [*Caloptenus spretus* Walsh] at Sacramento, California; and males and females reared from *M. atlanis* (Riley) at Brawley, California, and from unidentified grasshoppers at Alpine, California. Other specimens were from Lewiston and Moscow, Idaho; Pullman, Washington, and London, Ontario. A large number of females considered by Aldrich to be of the same species were from Trenton, Kentucky; these were collected in September by Ainslie, who observed them attacking flying grasshoppers.

A few first instar larvae that differed slightly from those of *A. aculeata* (63) were found in the dissections. These may be larvae of the variety *gavia* or *taediosa*. Apparently they are not of the closely related species *Acridiophaga caridei* (Brèthes) for they do not agree with the figures of *A. caridei* given by de Crouzel (29) and by Smith and Finlayson (63).

A. caridei has been reported in North America (15, 50, 51) and is considered one of the most important parasites of locusts in South America (3, 9, 46), but according to Lloyd (46) is singularly ineffective there against solitary species of grasshoppers.

Whether or not the South American species *A. caridei* (13) is synonymous with the North American species *Sarcophaga angustifrons* Ald. (2), as concluded by Aldrich (3) in 1927, or with specimens from North America identified as *A. caridei*, is of particular interest in view of the transfer of *A. caridei* from South America to Canada (46, 59). There is no evidence that larvae of *A. caridei* are present in Canadian grasshoppers.

Life History and Habits

Deposition of larvae by *A. aculeata* in the field was not observed. In cages the female strikes at moving grasshoppers and deposits one to several larvae at a time.

Mating occurs at any time after eclosion of the female. All larvae in a litter in the incubating uterus develop at the same rate. First instar larvae remain in a healthy condition in the uterus for as long as three weeks. It is probable that more than one litter is produced by a female during its lifetime. The number of larvae per female recorded below is the number present in one litter.

The first instar larva is metapneustic and the second and third are amphipneustic. The full-grown larva may continue development to the adult without interruption or may enter diapause. In the laboratory, larvae in diapause continued their development after exposure to a temperature of 8° C. for 67 days. Shorter periods at 8° C. or at lower temperatures would possibly be sufficient to break diapause.

Data on the life history of *A. aculeata* obtained from laboratory propagation are as follows:

	Max.	Min.	Mean
Number of larvae per female	121	10	44.0
Larval development in host (days)	26	4	9.0
Days ex host as mature larva	7	1	2.6
Days in puparium	17	11	12.9
Days from deposition of larva to adult	38	16	24.6
Gestation period, approximately (days)			9
Ratio of females to males	48.8 : 51.2		

The puparium was described and figured by Greene (36) and the somatic chromosomes were described by Boyes (11).

Seasonal Occurrence

Adults are present in the field from late June to late September with peaks of abundance in mid-July and mid-August. There are two or more generations per year.

Distribution

A. aculeata is widespread in North America. In Western Canada it was found from southeastern Manitoba to the dry interior of British Columbia and

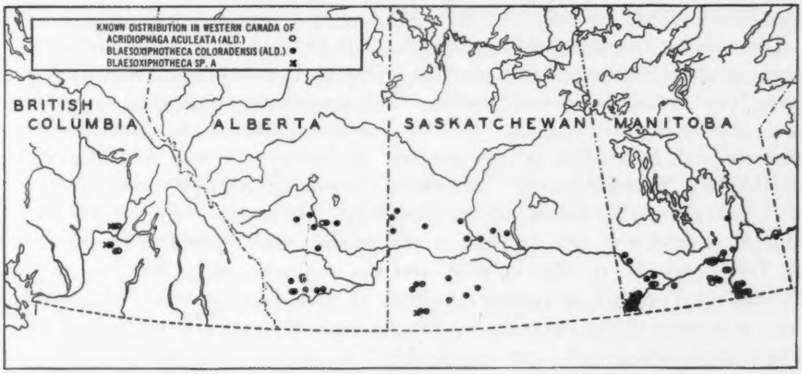


FIG. 1. Distribution of *Acridiophaga aculeata* (Ald.), *Blaesoxiphotheca coloradensis* (Ald.), and *Blaesoxiphotheca* sp. A in the four western provinces.

as far north as Winnipeg, Manitoba, and Drumheller, Alberta. It was not found in collections of grasshoppers from Saskatchewan but there is little doubt that it occurs in that province as well. The distribution (Fig. 1) makes no distinction between *A. aculeata* and its varieties.

Hosts

Amphitornus coloradus (Thos.), *Aulocara elliotti* (Thos.); *Camnula pellucida* (Scudd.); *Melanoplus bivittatus* (Say), *M. femur-rubrum* (DeG.), *M. mexicanus* (Sauss.), and *M. packardii* Scudd.

Incidence of Parasitism

Incidence of parasitism by this species rarely exceeded 5% and it rarely occurred in more than 2–3% of the collections of any one host species. However, an exceptionally high parasitism of 25.0% was found in one collection of 25 undetermined Acridinae collected in the Lac du Bois ranges, British Columbia, in late June, 1946. The incidence of the species as a grasshopper parasite was so limited that no satisfactory long-term average of parasitism could be established for any of the localities in which it was found.

Discussion

This species has been recorded as a parasite of grasshoppers (2, 15, 62), but there is little evidence that it is important. In one field in Ontario that was under observation for many years, females of this species, swollen with larvae, were conspicuously abundant at times, but grasshoppers in the same field were lightly parasitized by it. This low incidence of parasitism despite the presence of many gravid females suggests that *A. aculeata* is primarily a parasite of some other insect. It is known that it is not a scanvenger but requires a living host. In the laboratory it develops well in *C. pellucida*, *Dissosteira carolina* (L.), *M. femur-rubrum*, *M. mexicanus*, and *M. packardii*. The survival rate in *M. bivittatus* is low.

Blaesoxiphotheca coloradensis (Ald.)

This species was described by Aldrich (2) in 1916 as *Sarcophaga coloradensis* from specimens collected in Colorado. One male examined at the time was from New Bedford, Massachusetts. The species was not then recognized as a parasite of grasshoppers. In 1918 Townsend erected the genus *Blaesoxiphotheca* with *B. caudata* as type species; his holotype female was a paratype of Aldrich's *S. coloradensis*. Townsend considered Aldrich's male holotype of *S. coloradensis* to belong to *Acridiophaga*. However, there is little doubt that *S. coloradensis* Ald. belongs in the genus *Blaesoxiphotheca* as described by Townsend (77, p. 182) in 1938, and the similarity of its larva to that of *B. caudata* Tns. (63) is further evidence of their relationship. *Blaesoxiphotheca* was reported as parasitic on locusts by Townsend (78, p. 175) in 1935. The first references to *B. coloradensis* (Ald.) as a parasite of grasshoppers appear to be by Smith (62) in 1944 and by Buckell and Spencer (15) in 1945. The species was reared from grasshoppers in the present work as early as 1939, and its larvae were found in dissections in 1941 although at that time their identity was not recognized. Knipling (44) figured and described first instar larvae of this genus as early as 1936 and placed them in the "prohibita" group, a group that is frequently reared from insects.

Life History and Habits

B. coloradensis was not propagated in the laboratory and information on its life history and habits is limited. Up to 50 first instar larvae were found in an individual female. The larvae are probably placed on the host and gain entry in the same manner as those of *Acridiophaga* spp. If the long lateral spines of the first instar larvae of this genus (44, 63) have any functional importance in the behavior of the larvae it has not been discovered. When Knipling (44) attempted to rear larvae of this type on a pupa of *Hypoderma* sp. they began crawling posterior-end foremost through an opening made in the pupa, and could not be induced to travel anterior-end foremost. Nevertheless, it seems most likely that the larvae of *Blaesoxiphotheca* spp. gain entry with their mouth parts in the same manner as those of other sarcophagids.

The larvae are found in all stages of grasshoppers, but most often in those of the second to fifth instar. The mature larva pupates within a day or two after it leaves the host or it remains in diapause over winter. Larvae in diapause continued development after exposure to a temperature of 0° C. for 32 days, or to 8° C. for 46 days. Larvae were kept at 8° C. for 108 days without detriment to their subsequent development.

The first instar larva was described previously (63).

Seasonal Occurrence

Adults are present in the field from early June to late September with a peak of abundance in July. There are at least two generations per year.

Distribution

The species is widespread in Western Canada. It was found from south-eastern Manitoba to the interior of British Columbia and as far north as

Kamloops, British Columbia; Drumheller, Alberta; and Riverton, Manitoba (Fig. 1). It also occurs in Ontario and Quebec, and has been reported from Massachusetts (42) and Texas (51). It is probably generally distributed throughout temperate North America.

Hosts

Aeropedellus clavatus (Thos.); *Camnula pellucida*; *Melanoplus bivittatus*, *M. dawsoni* (Scudd.), *M. femur-rubrum*, *M. gladstoni* (Scudd.), *M. infantilis* Scudd., *M. mexicanus*, and *M. packardii*.

Incidence of Parasitism

Parasitism by *B. coloradensis* rarely exceeded 15%. The highest parasitism recorded, 48.3%, was found in *M. mexicanus* collected at Greenridge, Manitoba, June 23, 1941. The approximate proportions of collections with this parasite were as follows: *M. bivittatus*, 10%; *M. mexicanus*, 25%; *M. femur-rubrum*, 30%; and *M. infantilis*, 40%.

Discussion

Although the incidence of parasitism by *B. coloradensis* was usually not high, the species is relatively persistent in certain areas and must be considered an important early-season parasite. It accounted for as much as 35% of the total parasitism of *M. femur-rubrum* at Fortier, and as much as 40% of the total parasitism of *M. mexicanus* at Dominion City; both of these localities are in the Red River Valley of Manitoba. It was proportionately less important in southwestern Manitoba but accounted for 50% of the total parasitism of *M. infantilis* at Gravelbourg, Saskatchewan.

In Manitoba it is primarily a parasite of *M. mexicanus*, *M. femur-rubrum*, and *M. bivittatus*; in Saskatchewan, of *M. mexicanus* and *M. infantilis*; and in Alberta, of *M. mexicanus*, *M. femur-rubrum*, and *M. infantilis*. In British Columbia it was found only in *M. mexicanus*.

B. coloradensis itself suffers some mortality in its hosts. Of those found in *M. bivittatus*, 13.0% were dead; in *M. mexicanus*, 8.0%; in *M. femur-rubrum*, 7.0%; and in *M. infantilis*, 1.5%.

BLAESOXIPHOTHECA sp. A

Blaesoxiphotheca sp. A was recognized only in the first larval stage. It differs from that of *B. coloradensis*, which was described by Smith and Finlayson (63), by the less sicklelike oral hooks and by the narrower sinus between the dorsal and ventral arms of the pharyngeal skeleton (Fig. 2).

FIG. 2. Buccopharyngeal armature. A, *Blaesoxiphotheca coloradensis*. B, *Blaesoxiphotheca* sp. A. (After Smith and Finlayson (63).)

Life History and Habits

The species was not propagated in the laboratory and little is known of its biology. Its larvae were found in all host stages, but most frequently in third instar nymphs.

Seasonal Occurrence

The species is present in the field from early June to late August. The dates of occurrence of the first instar larvae suggest a peak of adult abundance in July. There are probably two generations per year.

Distribution

The species was found in British Columbia, and Manitoba (Fig. 1). It was not found in Alberta or, except for one questionable record, in Saskatchewan.

Hosts

Chorthippus longicornis (Latr.); ***Melanoplus bivittatus***, and ***M. mexicanus***.

Incidence of Parasitism

The species is rare in Manitoba, where it was found only in ***M. bivittatus*** and in less than 2.0% of the collections of that host. It was much more prevalent in British Columbia, where it was present in over 80.0% of the collections of ***M. mexicanus;*** maximum parasitism in this host was 12.0%.

BLAESOXIPHOTHECA sp. B

This species was recognized only in the first larval stage. The buccopharyngeal armature is similar to that of *Blaesoxiphotheca* sp. A (Fig. 2) but has shorter oral hooks. The larvae of the two species may be separated most readily by differences in the size, shape, and arrangement of the spines on the ventral surface between segments 4 to 11 (Fig. 3).

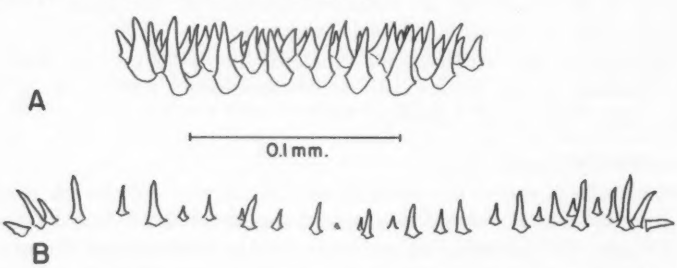


FIG. 3. Spines on venter between segments five and six of first instar larva. A, ***Blaesoxiphotheca*** sp. A. B, ***Blaesoxiphotheca*** sp. B.

The species was found only in ***M. bivittatus***, at Fortier, Manitoba. Five larvae were found in one male collected on July 17, 1944; three were found in two fourth instar nymphs and four in one female, all collected on July 29, 1953.

BLAESOXIPHOTHECA sp. C

Blaesoxiphotheca sp. C was recognized only in the first larval stage and is distinguished from other species of the genus by differences in the spine pattern

of the first instar larva. It differs from the previous three species in having the spines in the ventral band between segments three and four equal in length to those between segments four and five, rather than shorter. The size, shape, and arrangement of spines in the ventral bands between segments 4 to 11 are similar to those of *Blaesoxiphotheca* sp. B (Fig. 3).

All specimens of *Blaesoxiphotheca* sp. C occurred in *M. mexicanus*. Two larvae were found in a female collected at Granum, Alberta, August 30, 1944; one was found in a male collected on the Lac du Bois ranges, near Kamloops, British Columbia, July 29, 1945, and one in a fifth instar nymph collected at Asquith, Saskatchewan, June 17, 1949.

KELLYMYIA KELLYI (Ald.)

The male and female of this species were first described by Aldrich (1) in 1914 as *Sarcophaga kellyi*. Specimens examined at that time were from Texas, New Mexico, Arizona, Kansas, Colorado, Utah, Washington, and Manitoba. In 1916 Aldrich (2) noted that the specimens listed at the time of the original description were reared largely from grasshoppers. He subsequently examined specimens reared from adults of the wood-boring cerambycid beetle *Plectrodera scalator* (F.) found in cottonwood at Kinsley, Kansas, and from a tenebrionid beetle, *Asida* sp., taken at La Junta, Colorado. Townsend (72) erected the genus *Kellymyia* in 1917, with *S. kellyi* as type species.

At the time of Aldrich's original description in 1914, Kelly (43) reported on the species as a new sarcophagid parasite of grasshoppers. Kelly had noticed the flies striking at grasshoppers in Kansas in 1908 and 1912, and referred to reports of fly and maggot abundance in outbreaks of *Melanoplus differentialis* (Thos.) in Oklahoma in 1913. Smith (60) considered this species the most important factor in the control of *Dissosteira longipennis* (Thos.) in New Mexico in 1913. Buckell (14), reporting on the grasshopper outbreaks in British Columbia, noted that "the enormous increase in the numbers of *mexicanus* in 1943 and 1944 resulted in a species of sarcophagid, previously rare in British Columbia becoming very numerous in 1944, and it is due to the presence of this fly, *Sarcophaga kellyi* Ald., and some of its close allies, that there is reason to hope that the outbreak will soon be terminated".

There is little doubt that, when sarcophagid flies are abundant during grasshopper outbreaks, *K. kellyi* is usually the species to receive credit for reducing the grasshopper abundance.

Life History and Habits

In 1914, Kelly (43) stated that the species is viviparous and deposits its larvae on the undersides of the wings of flying grasshoppers. He also reported that the flies deposit larvae on the second, third, and fourth instar nymphs of *M. differentialis* and *M. bivittatus*, but only on those in motion. Smith (60), who observed this same species in New Mexico in 1913, observed larviposition throughout June. The females, as far as his observations went, always selected individuals of *D. longipennis* that were freshly molted or inactive but in a healthy condition. Smith also observed the sarcophagids larvipositing

on nymphs left comatose on the open prairie after being stung by the wasp *Priononyx atrata* Lep. In no instance did he see a sarcophagid attempt to strike a moving nymph or a flying adult. In one instance he observed a female larvipositing on a host that had dried out and hardened.

There is little agreement between the observations of Kelly and Smith on the method of attack by *K. kellyi* and it may be concluded that the parasite larviposits on both active and inactive living hosts, and on dead ones as well.

During the present study, *K. kellyi* was not observed attacking hosts in the field, but perhaps this could be observed readily only at times of grasshopper outbreaks. Limited tests in large field cages gave no evidence of successful attack on living grasshoppers, though in the laboratory this occasionally occurred. The flies larviposited freely on dead grasshoppers in the laboratory.

K. kellyi is the only species of those discussed in this paper that has been reared on other than living grasshoppers. It was rarely reared from collections of living grasshoppers, and on only one occasion were dead larvae, presumably of this species, found by dissection. This scarcity of *K. kellyi* may be explained by the fact that parasitized hosts become inert in as little as 24 hours after the entry of the parasite larvae, and thus may not be taken in sweep collections; or it may be that the species is largely saprophagous. Should the first explanation be correct, then the likelihood of finding living grasshoppers containing this parasite would not be great except under conditions of extreme host and parasite abundance.

K. kellyi can be propagated without difficulty in the laboratory. It was reared through 40 generations on pork liver. Larvae were also reared on dead grasshoppers, and on a mixture consisting of powdered milk, powdered egg, and brewers' yeast moistened with water until of the consistency of thick paste. The flies deposited their larvae freely on the liver and dead grasshoppers, but not on the mixture.

Mating occurs any time after eclosion of the adult. The incubating uterus may contain 20 to 60 first instar larvae at a time. At 25° C. the gestation period ranged from 8 to 60 days. As many as five litters of larvae were produced by a single female. The total number of larvae produced by a female no doubt depends upon the availability of food or hosts for larval deposition, for, in the absence of suitable food, larvae are retained by the female and the production of additional litters is delayed. When adequate food is available the interval between litters is approximately seven days.

Data on the life history of *K. kellyi* obtained from laboratory propagation at 24° to 28° C. are as follows:

	Max.	Min.	Mean
Number of larvae per female	262	26	133.0
Larval development in host (days)	6	2	3.3
Days in puparium	13	9	11.3
Days from deposition of larva to adult	21	14	16.5

The puparium is usually formed 1 to 2 days after the larva is full-grown.

At 24° to 28° C. development in the laboratory proceeds without interruption, and attempts to induce diapause by exposing mature larvae and pupae to temperatures of 8° and 0° C. were not successful and caused death. However, a small proportion of the mature larvae of the laboratory stock of the 34th to 36th generations remained in diapause, possibly as the result of a lowering of the rearing temperature to 21° C. for 1 week. Adults did not survive prolonged periods at 8° C. but lived as long as 150 days at 24° C. One female at 90 days of age produced viable larvae. The first instar larva was described by Knipling (44) and by Smith and Finlayson (63). The puparium was figured and described by Greene (36), and the somatic chromosomes were studied by Boyes (11).

Seasonal Occurrence

Adults of *K. kellyi* are present in the field from mid-June to mid-October, and possibly until the first severe frost. Because of the occasionally long gestation period and the absence of a marked diapause it is difficult to estimate the number of generations per year. It is presumably from two to four. The winter is apparently passed in the soil in the mature larval stage since the adult and pupal stages do not survive prolonged periods of exposure at temperatures of 8° C. or lower.

Distribution

The species was found from Lyleton, Manitoba, in the east, to Kamloops, British Columbia, in the west, and from Dollard, Saskatchewan, in the south, to Saskatoon, Saskatchewan, in the north (Fig. 4). Aldrich (2) stated that no specimens of *K. kellyi* were seen from any locality east of central Kansas.

Hosts

In the almost complete absence of *K. kellyi* in the rearings and dissections of field-collected grasshoppers, the investigations provided little information on the probable hosts of this sarcophagid. It has been reported as a parasite

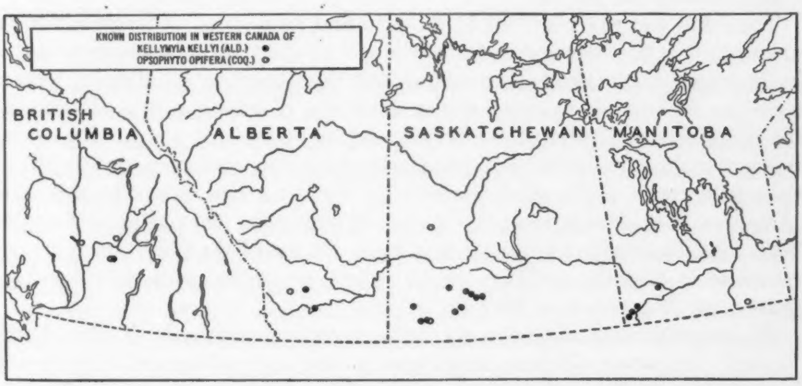


FIG. 4. Distribution of *Kellymyia kellyi* (Ald.) and *Opsophyto opifera* (Coq.) in the four western provinces.

of *M. bivittatus* (43), *M. mexicanus* (15), and *D. carolina* (60), and it was propagated in the laboratory on *C. pellucida, D. carolina, M. bivittatus,* and *M. packardii.* It was propagated on liver and has been taken in traps baited with lean beef (51). The great variety of foods suitable for its development suggests that all grasshopper species are potential hosts, though the parasite's ability to infest active hosts remains questionable.

Incidence of Parasitism

No records on the incidence of parasitism by *K. kellyi* were obtained, though it has frequently been reported in the literature as bringing outbreaks of grasshoppers under control.

The almost complete absence of *K. kellyi* in collections of living grasshoppers may be due either to the rapidity with which infested grasshoppers are immobilized or to the fact that the parasite is not particularly effective except in grasshopper outbreaks, when, because of its rapid larval development and semisaprophagous habit, it is able to take advantage of the abundance of molting, weakened, and dying hosts.

OPSOPHYTO OPIFERA (Coq.)

Opsophyto opifera was described by Coquillett (24) as *Sarcophaga opifera* in 1892, when it was found parasitizing *Melanoplus devastator* Scudd. in California. In 1915, Townsend (70) designated it as the type of his genus *Opsophyto.* When Aldrich (2) redescribed the species in 1916 he examined specimens from California, Washington, Idaho, and Texas. These were reared from *M. devastator, M. marginatus* (Scudd.), *M. plumbeus* (Dodge), and *M. bivittatus.* Treherne and Buckell (80) reported it as common in the Chilcotin district of British Columbia in 1920 and 1921, and in the lower Okanagan Valley in 1923. It was reared at Belleville from *M. femur-rubrum* collected at St. Isidore and St. Jean, Quebec, in 1940, and at Charlottetown, Prince Edward Island, in 1949.

Life History and Habits

The adult was not observed in the field and its method of larviposition is unknown. It deposits first instar larvae and as many as 57 larvae were found in the uterus at one time. It was reared in the laboratory by allowing larvae from gravid females to enter the severed legs of grasshoppers. However, the sluggish movements of the larvae and their habit of contracting when touched makes this method of propagation less satisfactory with *O. opifera* than with many of the other parasites. The few larvae reared in this way completed their development in 7 days, and pupated 2 days after leaving the host. Adults emerged 12 to 13 days later. Some of the fully grown larvae remained in diapause at 25° to 26° C. These proceeded with their development after an exposure to 8° C. for 77 days.

All nymphal and adult stages of grasshoppers are parasitized. Treherne and Buckell (80) reported larvae of this species feeding upon eggs of *C. pellucida* in the Nicola Valley, British Columbia, in May, 1923.

The first instar larva (63) and the puparium (36) have been figured and described.

Seasonal Occurrence

Larvae of *O. opifera* were found in grasshoppers collected as early as June 14 and as late as September 14. Maximum abundance of *O. opifera* in British Columbia occurred in late July and in August. There are probably two summer generations and an overwintering one.

Distribution

O. opifera was found in the four western provinces but was most abundant in British Columbia. It was not found by dissection in grasshoppers from the three Prairie Provinces, but adults were collected at Stavely, Alberta, and at Herschel, and Shaunavon, in Saskatchewan; it was reared from grasshoppers collected at Greenridge, Manitoba (Fig. 4).

Hosts

O. opifera was reared from *C. pellucida* and *M. mexicanus*, and found by dissection in these hosts, and in *M. confusus* Scudd. and *A. coloradus*. A few *O. opifera* found in *M. bivittatus* were dead in the first larval stage, indicating that it does not survive well in this host, though it has been reared from it (2).

Incidence of Parasitism

The species appears to be an important parasite only in British Columbia, though present in the other three provinces. Over 40% of the collections of *M. mexicanus* from the Lac du Bois ranges, British Columbia, contained this parasite but the degree of parasitism for the years 1945 and 1946 was low; maximum parasitism recorded was 5.0%. Considering the wide yearly fluctuations in parasitism by other species, it may possibly be that this does not represent the long-term average condition. In 1953, a number of *O. opifera* were reared by E. Peters from *C. pellucida* collected in the Lac du Bois ranges in early July; the estimated incidence of parasitism was 5%. *O. opifera* was not found in the dissections of grasshoppers from any other province, and it may be concluded that it contributes little to the natural control except in British Columbia.

PROTODEXIA HUNTERI (Hough)

The species was described by Hough (40) in 1898 as *Sarcophaga hunteri* from specimens reared from *M. differentialis* in Kansas. The genus *Protodexia* was proposed by Townsend (68) in 1912 with the holotype female *P. synthetica* from Melrose Highlands, Massachusetts, and the genus was further elucidated by him (71) in 1916. In 1938, Townsend (78, p. 90) concluded that *P. synthetica* was synonymous with *S. hunteri*, which now becomes the type species for *Protodexia*. In 1947, Reinhard (56) described a new subspecies, *S. hunteri aenigma*, from Long Island, New York.

Morgan (48) listed *P. hunteri* as a parasite of *M. differentialis* in the Mississippi Delta in 1901, and Kelly (43) reported it as a parasite of *Chortophaga viridifasciata* (DeG.), *M. bivittatus*, and *M. differentialis* at Wellington, Kansas, in 1913. Aldrich (2), in 1916, examined specimens from British Columbia, Idaho, Utah, California, New Mexico, Arizona, Kansas, Nebraska, Missouri, Ohio, Pennsylvania, New Jersey, and Massachusetts. Treherne and Buckell

(80) stated that it was common on the ranges around Vernon and Kamloops, British Columbia. Cole and Lovett (23) listed it from Oregon, Johnson (42) from New England, and Hallock (37) from New York. It was taken in fly traps in Texas by Parish and Cushing (51). Several representatives of the genus occur in South America (46).

Life History and Habits

P. hunteri is considered larviparous, though the first instar larva is enclosed in a thin, membranous envelope or chorion when deposited. The gravid female either trails the prospective host patiently or dances excitedly about it awaiting a favorable opportunity to deposit its larvae. Eventually the fly carefully approaches the grasshopper, brings the tip of the abdomen forward between its legs, and tucks a larva into some fold on the grasshopper's body. This is done in a deliberate manner and it seems unlikely that *P. hunteri* would attack flying grasshoppers as reported by Parker (2, postscript). The larva is dorsoventrally flattened and well suited to its manner of placement. The larva begins to work its way into the host almost immediately.

Fifth instar and adult grasshoppers are the stages usually parasitized, fourth instars rarely.

Mating of the adults occurs any time after eclosion. As many as 88 larvae were found in an individual female.

Data on the life history of *P. hunteri* obtained from propagation in the laboratory at 24° C. are as follows:

	Max.	Min.	Mean
Number of larvae per female	88	8	34.1
Larval development in host (days)	18	4	8.3
Days ex host as mature larva	21	1	1.9
Days in puparium	46	11	14.6
Days from deposition of larva to adult	54	18	23.0
Gestation period, approximately (days)			9
Ratio of females to males		51.7 : 48.3	

Late-season larvae frequently entered diapause. Such larvae proceeded with their development after exposure to a temperature of 8° C. for 50 days, and some larvae exposed to this temperature for as many as 150 days successfully completed their development.

The first instar larva was figured and described previously by Smith and Finlayson (63), and the puparium by Greene (36). The genitalia were figured by Hallock (37, 38), the somatic chromosomes by Boyes (11).

Seasonal Occurrence

P. hunteri is present in the field from early June until late September, and probably until the first severe frost. There are two summer generations and an overwintering one.

Distribution

The species is widespread and occurs from Tolstoy in southeastern Manitoba to Kamloops in British Columbia. The known northern limits of its dis-

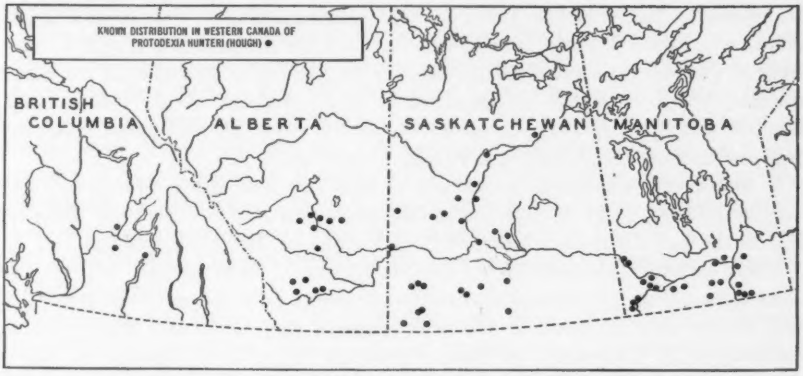


FIG. 5. Distribution of *Protodexia hunteri* (Hough) in the four western provinces.

tribution are Morrin, Alberta; White Fox, Saskatchewan, and Wattsview, Manitoba.

Hosts

Aeropedellus clavatus, *Ageneotettix deorum* (Scudd.), *Amphitornus coloradus*, *Aulocara elliotti*; *Camnula pellucida*; *Melanoplus angustipennis* (Dodge), *M. bivittatus*, *M. confusus*, *M. dawsoni*, *M. femur-rubrum*, *M. infantilis*, *M. mexicanus*, and *M. packardii*.

Incidence of Parasitism

The highest parasitism recorded for *P. hunteri*, 36.1%, was found in *M. packardii* collected at Rainbow, Alberta, in 1950. The approximate proportions of collections with this parasite were as follows: *C. pellucida*, 8%; *M. bivittatus*, 8%; *M. mexicanus*, 15%; and *M. packardii*, 25%.

The annual series of collections from British Columbia were small and serve little more than to indicate the occurrence of the parasite in that province.

Discussion

P. hunteri is primarily a parasite of fifth instar and adult grasshoppers. Maximum parasitism occurs from late July to early September but is sufficiently early to effect a reduction in the deposition of grasshopper eggs.

The species is of little importance in the Red River Valley of Manitoba but becomes increasingly abundant in southwestern Manitoba, Saskatchewan, and Alberta.

M. packardii and *M. mexicanus* are among the preferred hosts. Although *M. bivittatus* was frequently attacked, larvae of *P. hunteri* seldom survived beyond the first larval instar in it.

SARCOPHAGA FALCIFORMIS Ald.

This species was described by Aldrich (2) in 1916. The holotype male was reared from *M. devastator* at Eureka, California. Included among the specimens examined by Aldrich was one male from Aweme, Manitoba, reared from

M. atlanis by N. Criddle on July 25, 1913. Other specimens were from Illinois, Wisconsin, South Dakota, and Colorado. A teneral female bearing the same label as the holotype male was considered by Aldrich as probably of the same species though scarcely distinguishable from females of *B. coloradensis.* Buckell and Spencer (15) reared *S. falciformis* from *M. mexicanus* collected between Lytton and Kamloops, British Columbia.

S. falciformis was taken in sweeps at Sperling, and Elva, Manitoba, and was reared from acridids collected at Greenridge, Manitoba; Stavely, Alberta; and in the Lac du Bois ranges, north of Kamloops, British Columbia.

There is little information on the life history of this species. Specimens were reared from grasshoppers collected between July 20 and September 15, and adults were captured in the field between August 15 and September 4. As Aldrich (2) has stated, the female resembles very closely that of *B. coloradensis.* It may be distinguished from the latter, however, on the relative length of the sternites of the seventh and eighth segments as they occur in the sternotheca. There is also a difference in the density and distribution of the bristles on the sternite of the seventh segment (Fig. 6).

No larvae found in dissections were recognized as belonging to this species but possibly those considered as one of the species of *Blaesoxiphotheca* discussed above are of this species.

The small number of specimens reared from grasshoppers would suggest that this species is not of great economic importance in the natural control of grasshoppers. Maximum incidence of parasitism was 4.0%, and the percentage of collections to yield this species was extremely small.

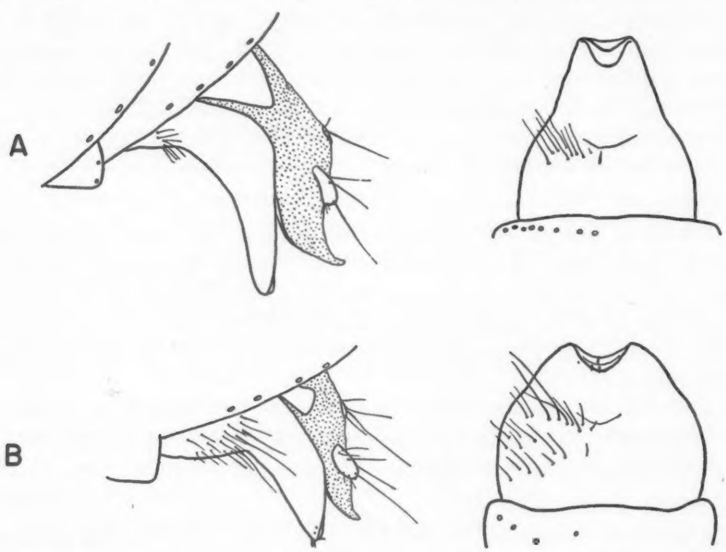


FIG. 6. Lateral and ventral view of sternotheca of female. A, *Blaesoxiphotheca coloradensis* (Ald.). B, *Sarcophaga falciformis* Ald.

Sarcophaga reversa Ald.

This species was described by Aldrich (2) in 1916; the holotype male was from LaFayette, Indiana. Other males examined at the time were from Massachusetts, New Jersey, Pennsylvania, New York, Quebec, California, Idaho, Colorado, Washington, and South Dakota. The species has also been recorded from New York (37, 45). Hayes and DeCoursey (39) found it as a parasite of grasshoppers at Urbana, Illinois, in 1937. This species and *Sarcotachinella sinuata* were reared by them from grasshoppers collected alive. In view of its widespread occurrence in grasshoppers in Canada it is curious that Hayes and DeCoursey should be the first to record *S. reversa* as a parasite of these insects.

Present studies indicate that *S. reversa* and three closely related species (or subspecies) constitute a group of major importance as parasites of nymphal and adult grasshoppers. These are: *S. reversa* and *Sarcophaga* sp. C, sp. E, and sp. H, of Smith and Finlayson (63) distinguished by differences in the first instar larvae. No characters were found to distinguish the adults of *S. reversa* from those of *Sarcophaga* sp. H. Adults of *Sarcophaga* sp. C can be distinguished on genitalic characters (Fig. 8). Adults of *Sarcophaga* sp. E are as yet unrecognized.

Life History and Habits

In the laboratory the fly runs over a grasshopper or alights on it momentarily to deposit its larvae, one or more at a time. The larvae enter freshly-molted hosts, and younger nymphs, more quickly than they do the older ones.

As many as 80 first instar larvae were found in a female. It was not determined whether a female produces more than one litter.

Full-grown larvae either pupate and proceed with their development a day or two after leaving the host or remain as larvae in diapause. Diapause larvae proceeded with their development after exposure to a temperature of 0° C. for 32 days or to 8° C. for 67 days. Exposure to these temperatures for as many as 200 days had no apparent harmful effect.

In the field, adults were observed feeding on the honeydew from aphids infesting *Artemisia cana* Pursh.

Data on the life history of *S. reversa* as determined from propagation in the laboratory at 24° C. are as follows:

	Max.	Min.	Mean
Number of larvae per female	81	6	41.3
Larval development in host (days)	24	8	11.7
Days ex host as mature larva	10	1	2.7
Days in puparium	15	9	11.0
Days from deposition of larva to adult	36	20	24.8
Gestation period, approximately (days)			9
Ratio of females to males		43.0 : 57.0	

Smith and Finlayson (63) figured and described the first instar larva and Boyes (11) the somatic chromosomes.

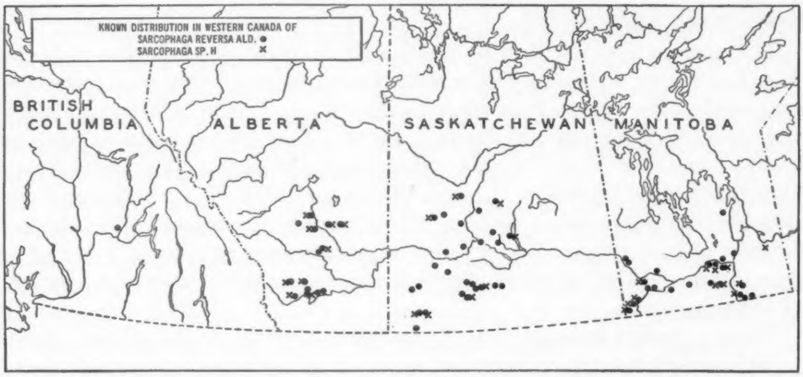


FIG. 7. Distribution of *Sarcophaga reversa* Ald. and *Sarcophaga* sp. H in the four western provinces.

Seasonal Occurrence

Adults of *S. reversa* are present in the field from early June to early September with a peak of abundance in July. There is one, and possibly a second summer generation, and an overwintering one.

Distribution

The species is widespread and was found from Tolstoy, southeastern Manitoba, through southern Saskatchewan to Lethbridge, Alberta. It occurs as far north as Drumheller, Saskatoon, and Winnipeg. It was not found in dissections of grasshoppers from British Columbia but was reported as a parasite of *M. mexicanus* in that province by Buckell and Spencer (15). It was reared during the present study from grasshoppers collected north of Kamloops in the Lac du Bois ranges.

The failure to distinguish regularly between the larvae of *S. reversa* and of *Sarcophaga* sp. H prior to 1947, and the present inability to separate adults of these two species, restricts the usefulness of some of the rearing and early dissection records as sources of information on distribution. Adults of both these species, and perhaps also those of *Sarcophaga* sp. C of Smith and Finlayson (63), run to *S. reversa* in existing keys.

Hosts

Aeropedellus clavatus; Camnula pellucida; Melanoplus angustipennis, M. bivittatus, M. dawsoni, M. confusus, M. femur-rubrum, M. mexicanus, M. packardii, and *Phoetaliotes nebrascensis* (Thos.). *C. pellucida* is included in the list on the basis of a single occurrence. Failure to separate *S. reversa* from *Sarcophaga* sp. H in the early records makes the following list less certain: *Ageneotettix deorum, Aulocara elliotti, Chorthippus longicornis, Melanoplus infantilis,* and *Orchelimum* sp.

Incidence of Parasitism

This species is one of the most important of the sarcophagid parasites of grasshoppers in Western Canada; others of importance are *Sarcophaga* sp. H and *Sarcophaga* sp. C, and possibly *B. coloradensis*.

The highest parasitism recorded for *S. reversa*, 47.3%, was found in *M. bivittatus* collected at Lyleton, Manitoba, July 11, 1951. The approximate proportions of collections with this parasite were as follows: *M. mexicanus*, 7%; *M. packardii*, 9%; *M. femur-rubrum*, 10%; and *M. bivittatus*, 20%.

Discussion

S. reversa occurs relatively early in the season. It attacks all host stages but most frequently those of the third to fifth instars. Like many other sarcophagids, it suffers considerable mortality in *M. bivittatus*—up to 60.0% in some instances. Nevertheless, it appears to have a preference for this host when present, particularly in Manitoba. *S. reversa* decreases in relative importance from Manitoba westward and its place is taken by *Sarcophaga* sp. H, and *Sarcophaga* sp. C (Table I).

The great fluctuations in the annual abundance of these three species suggest a sensitivity to some variable in the environment.

SARCOPAHGA sp. H

Sarcophaga sp. H runs to *S. reversa* in keys by Aldrich (2) and Hallock (37), and at present, no reliable characters for the separation of the adults of these two species are known. The species are distinguishable in the first larval instar (63), that of *S. reversa* has the middorsal section of the third and fourth segments apparently without spines; spines on upper half of fifth segment are in dorsolateral patch only, and spineless middorsal section of fifth segment is equal in length to four or five times that of longest spines of the lateral patch. The first instar larva of *Sarcophaga* sp. H has the spines on the third and fourth segments dense almost to the middorsal line, and the fifth segment encircled with spines except for middorsal section which rarely exceeds length of longest spines in dorsolateral group. The oral hooks of *Sarcophaga* sp. H are usually longer and less robust than those of *S. reversa*.

Life History and Habits

The life history and behavior are similar to those of *S. reversa*.

The species was propagated in the laboratory on living grasshoppers, both by allowing the larvae to penetrate the integument in the normal manner, and by placing larvae from gravid females on the severed ends of host femora.

Data on the life history of *Sarcophaga* sp. H as determined by propagation in the laboratory at 24° C. are as follows:

	Max.	Min.	Mean
Number of larvae per female	79	9	35.8
Larval development in host (days)	33	7	13.9
Days ex host as mature larva	16	3	5.6
Days in puparium	16	7	12.8
Days from deposition of larva to adult	46	22	33.1
Gestation period, approximately (days)			9
Ratio of females to males		47.0 : 53.0	

The full-grown larva, like that of the previous species, either pupates and proceeds with its development to the adult or enters diapause in the larval stage. As few as 30 days at a temperature of 0° C., or 50 days at 8° C. were sufficient to satisfy the diapause requirement. Larvae held at a temperature of 0° C. for 55 days and then at 8° C. for 140 days pupated and produced healthy adults. Prior to cold treatment, larvae remained in diapause at 24° C. for as long as 83 days.

Larval development is slower in younger hosts. The mean time for development of the parasite when fourth instar, fifth instar, and adult hosts were used was 18.0, 16.0, and 13.9 days, respectively. In some instances, parasites were still in the first larval stage 6 days after entering the host.

All nymphal and adult stages of the host are attacked, but the parasite was found most frequently in third to fifth instar grasshoppers.

Adults were observed feeding in the field on the flowers of mustard and snowberry, and on the honeydew of aphids infesting *A. cana*.

The first instar larva was described and figured by Smith and Finlayson (63) and the somatic chromosomes were figured and described by Boyes (11).

Seasonal Occurrence

Sarcophaga sp. H is present in the field from early June to late September with a peak in late July and early August. It is somewhat later than *S. reversa*, though both species occur together.

There is one, and a partial second, summer generation, and an overwintering one. Approximately 40.0% of the larvae of the first summer generation and about 85.0% of the second enter diapause.

Distribution

Sarcophaga sp. H is widespread and occurs from southeastern Manitoba through southern Saskatchewan to Granum in Alberta. It occurs as far north as Drumheller, Saskatoon, and Winnipeg (Fig. 7).

Hosts

Aeropedellus clavatus, *Aulocara elliotti*; *Camnula pellucida*; *Melanoplus bivittatus*, *M. borealis junius* (Dodge), *M. confusus*, *M. dawsoni*, *M. femur-rubrum*, *M. gladstoni*, *M. infantilis*, *M. mexicanus*, *M. packardii*, and *Phoetaliotes nebrascensis*.

Incidence of Parasitism

Sarcophaga sp. H is one of the three most abundant sarcophagid parasites of grasshoppers in Western Canada. Hosts particularly subject to attack by it are *M. mexicanus*, *M. femur-rubrum*, and *M. packardii*. *S. reversa*, on the other hand, parasitizes *M. bivittatus* most heavily.

The highest parasitism recorded for *Sarcophaga* sp. H., 52.9%, was found in *M. mexicanus* collected at Glenbain, Saskatchewan, July 23, 1952. The approximate proportions of collections with this species were as follows: *M. bivittatus*, 20%; *M. femur-rubrum*, 40%; *M. packardii*, 40%; and *M. mexicanus*, 45%.

Discussion

Sarcophaga sp. H was of particular importance in south central Saskatchewan as a parasite of *M. mexicanus*. It effected as much as 80.0% of the total parasitism in collections of this species taken in Saskatchewan from mid-July to mid-August. As with *S. reversa*, the degree of parasitism fluctuated widely, and suggests a sensitivity of the parasite to one or more variables in the environment.

SARCOPHAGA sp. C

Adults of *Sarcophaga* sp. C run to *S. reversa* in published keys (2, 36), but can be distinguished from *S. reversa* on genitalic characters (Fig. 8). There are also distinctive characters in the first larval instar (63).

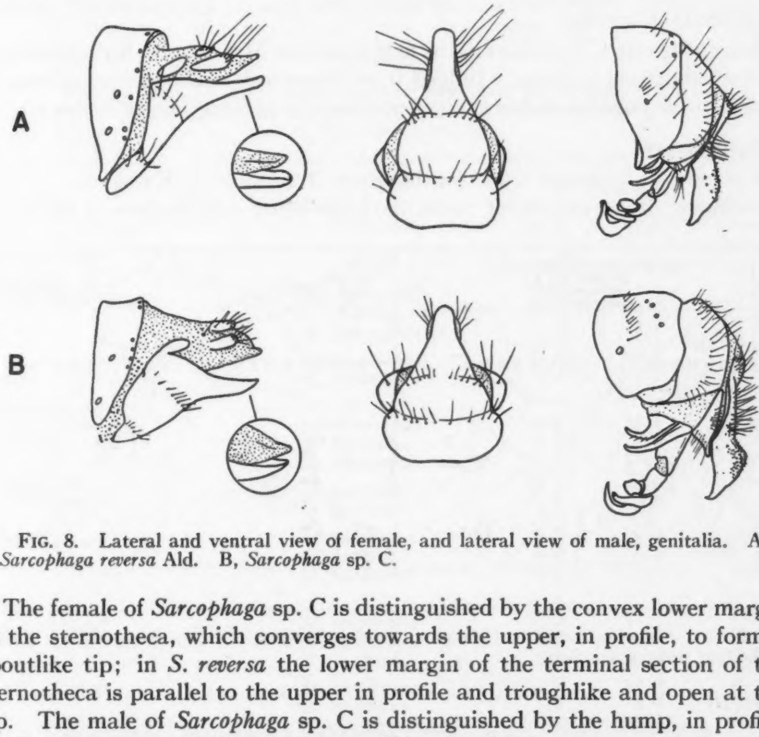


FIG. 8. Lateral and ventral view of female, and lateral view of male, genitalia. A, *Sarcophaga reversa* Ald. B, *Sarcophaga* sp. C.

The female of *Sarcophaga* sp. C is distinguished by the convex lower margin of the sternotheca, which converges towards the upper, in profile, to form a spoutlike tip; in *S. reversa* the lower margin of the terminal section of the sternotheca is parallel to the upper in profile and troughlike and open at the tip. The male of *Sarcophaga* sp. C is distinguished by the hump, in profile, on the hind margin of the anal forceps (Fig. 8). In *S. reversa* the hind margin is more gently curved.

S. reversa, *Sarcophaga* sp. H, and *Sarcophaga* sp. C are evidently very closely related, but differences in the larvae, in regional abundance, and in host preferences make it advisable to treat each as a valid species until more is known of their relationships. Individuals of *Sarcophaga* sp. C, both larvae and adults, are usually the most robust of the three in general form.

Life History and Habits

As many as 58 first instar larvae were found in an individual female, and one female remained alive for 193 days at 24° C. on a diet of water and cube sugar.

Under laboratory conditions the female carefully approaches a grasshopper and when sufficiently close hops onto it and deposits a larva. If the grasshopper is small she may grasp it firmly in her legs to place a larva, rolling over in the process.

All nymphal and adult stages of grasshoppers are attacked, second to fifth instar nymphs most frequently and adults rarely. Larval development is completed in 12 to 26 days and the fully grown larva leaves the host for pupation.

Seasonal Occurrence

Sarcophaga sp. C is present in the field from late May to mid-September and is most abundant in June. Judged from laboratory rearings, approximately 60.0% of the population has two generations a year, and the remainder one.

Distribution

The species is found from southeastern Manitoba to Kamloops, British Columbia. It occurs as far north as Drumheller and Saskatoon (Fig. 9).

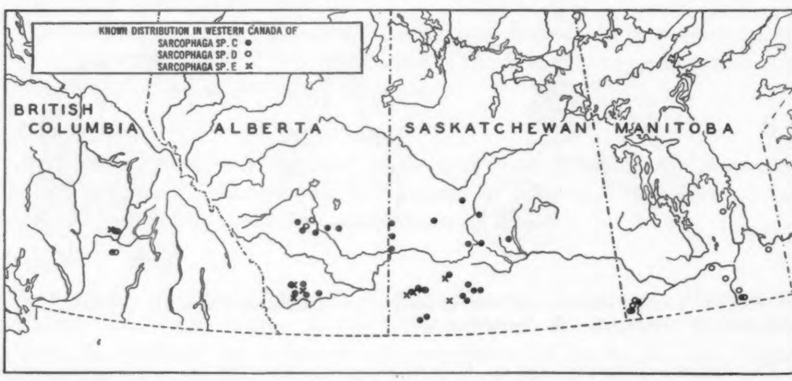


FIG. 9. Distribution of *Sarcophaga* sp. C, *Sarcophaga* sp. D, and *Sarcophaga* sp. E in the four western provinces.

Hosts

Aeropedellus clavatus, *Amphitornus coloradus*, *Chorthippus longicornis*; *Camnula pellucida;* *Melanoplus bivittatus*, *M. dawsoni*, *M. femur-rubrum*, *M. gladstoni*, *M. infantilis*, *M. mexicanus*, and *M. packardii.*

Incidence of Parasitism

The species is an important parasite of *M. mexicanus*. It is rare in Manitoba but relatively abundant further west. Highest parasitism by *Sarcophaga* sp. C, 43.2%, was found in *M. mexicanus* collected in the Lac du Bois ranges north

of Kamloops, June 15, 1946. The approximate proportions of collections with this species were as follows: *M. bivittatus*, 3%; *M. packardii*, 8%; and *M. mexicanus*, 28%.

Discussion

During the years of investigation, *Sarcophaga* sp. C was an important parasite of *M. mexicanus* in Saskatchewan, Alberta, and British Columbia. It was the only species of the *S. reversa* group found by dissection in grasshoppers collected in British Columbia in 1945 and 1946, though *S. reversa* (or *Sarcophaga* sp. H) was reared from grasshoppers collected in the Lac du Bois ranges in 1950 and 1951.

Differences in the regional importance and in the host preferences of *S. reversa*, *Sarcophaga* sp. H, and *Sarcophaga* sp. C are shown in Table I.

TABLE I

PERCENTAGES OF TOTAL PARASITISM OF ACRIDIDAE BY *Sarcophaga* sp. C, *Sarcophaga* sp. H, and *Sarcophaga reversa* IN VARIOUS AREAS IN WESTERN CANADA

Area	Host	Parasite		
		sp. C	sp. H	*reversa*
Manitoba				
Red River Valley[1]	*M. mexicanus*	0.0	11.4	4.2
	M. bivittatus	0.2	1.4	31.5
	M. femur-rubrum	0.0	35.8	5.2
Southwestern[2]	*M. mexicanus*	0.6	55.7	0.6
	M. bivittatus	0.5	7.2	50.6
Saskatchewan				
South central[3]	*M. mexicanus*	6.0	55.4	0.9
	M. bivittatus	0.0	2.5	7.4
	M. packardii	1.2	27.9	2.3
Dollard	*M. mexicanus*	21.0	14.0	0.0
	M. bivittatus	4.4	0.0	0.0
West central[4]	*M. mexicanus*	25.9	13.0	2.9
	M. bivittatus	0.0	4.7	28.1
	M. packardii	1.0	0.0	4.8
Alberta				
Carmangay[5]	*M. mexicanus*	33.3	42.4	0.5
	M. bivittatus	2.5	11.4	0.6
	M. packardii	1.8	29.3	2.2
Drumheller[6]	*M. mexicanus*	33.8	19.9	0.3
	M. bivittatus	1.4	2.6	2.8
	M. femur-rubrum	4.3	35.7	0.0
British Columbia				
Lac du Bois[7]	*M. mexicanus*	74.8	0.0	0.0
Nicola Valley[8]	*M. mexicanus*	43.8	0.0	0.0

[1]Arnaud, Dominion City, Fortier, Sperling. [2]Elva, Lyleton, Melita. [3]Glenbain, Gravelbourg, Hodgeville. [4]Asquith, Herschel. [5]Carmangay, Granum, Stavely. [6]Carbon, Munson, Nacmine, Rainbow. [7]Lac du Bois ranges, about 15 miles northwest of Kamloops, 2200 to 3200 ft. elevation. [8]Nicola Valley ranges, about 25 miles south of Kamloops, 2200 to 4500 ft. elevation.

SARCOPHAGA sp. D

Sarcophaga sp. D of Smith and Finlayson (63) was recognized only in the larval stage. Little is known of the biology of the species but it is assumed to be similar to that of *S. reversa*. One feature of *Sarcophaga* sp. D is its almost complete restriction to the Acridinae. All stages of the hosts are parasitized.

Larvae differing slightly in the size and the abundance of spines from those described as *Sarcophaga* sp. D by Smith and Finlayson (63), but with similar distribution and host preferences, have been considered as of that species in the account below.

Seasonal Occurrence

Sarcophaga sp. D is present in the field from early June to late August, with peak abundance in July.

Distribution

In Manitoba, the species was found from Dominion City in the south to Hodgson in the north. It was not recorded from Saskatchewan but occurred in the Drumheller area of Alberta, and in the Lac du Bois and Nicola Valley ranges in British Columbia (Fig. 9). Its occurrence in the Drumheller area of Alberta is based on the finding of two larvae, one at Orkney in 1946 and one at Carbon in 1952. Its occurrence in British Columbia is based on larvae found in grasshoppers collected in 1946; one larva was found in the Nicola Valley and three were found in the Lac du Bois ranges.

Hosts

Aeropedellus clavatus, Chorthippus longicornis; *Melanoplus bivittatus*, and *M. mexicanus*. There are many records of its occurrence from unidentified Acridinae.

Incidence of Parasitism

The general level of parasitism of the Acridinae by *Sarcophaga* sp. D rose from a low of 0.5% in early June to a high of 3.0% in late July. It declined through August and there was none in September. Maximum parasitism, 15.0%, was found in Acridinae collected at Fortier, Manitoba, July 23, 1949.

The usually low level of parasitism and low frequency of occurrence of *Sarcophaga* sp. D in the collections indicate that it is not a parasite of great importance, although it accounted for 20.0 to 50.0% of the total parasitism in the Acridinae.

SARCOPHAGA sp. E

Sarcophaga sp. E was recognized in the first larval stage only (63). It was not found in Manitoba and was of limited occurrence in the other western provinces. It was found in southern Saskatchewan, southern Alberta, and the Lac du Bois ranges north of Kamloops, British Columbia (Fig. 9).

Though not a species of importance, it occurred frequently enough to merit mention. Its preferred hosts are *M. packardii* and *M. mexicanus*; it was found

occasionally in *M. bivittatus*, once in *Amphitornus coloradus*, and once in an unidentified Oedipodinae. It occurred in all host stages but was most common in the third and fourth instars.

Maximum parasitism, 14.3%, was found in *M. packardii* collected at Stavely, Alberta, June 13, 1946.

SARCOTACHINELLA SINUATA (Mg.)

This species was originally described by Meigen in 1826 as *Sarcophaga sinuata* from specimens collected in Germany. The generic name *Sarcotachinella* was used with the trivial name *intermedia* by Townsend in 1892 to describe a sarcophagid collected in Illinois. *S. intermedia* was treated as a synonym of *S. sinuata* by Aldrich (2) in 1916. Later, in 1937, Townsend (77, p. 215) listed *S. sinuata* as the type species of *Sarcotachinella*.

It has been reared in British Columbia from *M. mexicanus* by Buckell and Spencer (15), and from *M. bivittatus* by Dr. R. D. Bird (in litt.), Entomology Laboratory, Brandon, from specimens collected at Arnaud, Manitoba. Kelly (43) reported it as a parasite of *M. differentialis*, and Hayes and DeCoursey (39) reared it from unidentified grasshoppers collected alive in Illinois. Aldrich (2) states in a postscript to his paper of 1916, that *S. sinuata* was observed attacking grasshoppers in flight.

S. sinuata was neither reared nor dissected from grasshoppers during the present investigation but adults of the species were commonly taken in sweeps. The larvae of *S. sinuata* are similar to those of *K. kellyi* in many respects and if the two species have similar habits it may explain the absence of *S. sinuata* in both rearings and dissections of grasshoppers.

Seasonal Occurrence

On the basis of captures, the flies are in the field from early June to late August.

Distribution

The species occurs from eastern Manitoba to British Columbia. Aldrich (2) listed it as occurring from Massachusetts to Colorado and Idaho. Cole and Lovett (23) recorded it from Oregon. Its occurrence in Germany indicates a holarctic distribution.

Discussion

In the absence of data on the incidence of this species it is not possible to estimate its importance as a natural control factor.

TEPHROMYIELLA ATLANIS (Ald.)

This species was described by Aldrich (2) as *Sarcophaga atlanis* in 1916. The holotype male and allotype female were reared from *M. mexicanus* collected at Franklin, New Hampshire. In 1918, Townsend (74) described the species as *Tephromyiella frankliniana*, using the allotype female of *S. atlanis* as his holotype female and as type species. However, Townsend (78, pp. 94–95) later decided that the species were conspecific thus making *S. atlanis* the type species of *Tephromyiella*.

This was the species reported by Marlatt (47) in 1889 as present in 5% of the grasshoppers in an outbreak at Franklin, New Hampshire. Other specimens examined by Aldrich (2) were from Sandusky, Ohio, and Aberdeen, South Dakota. Johnson (42) recorded the species from New England, and Hallock (37) recorded it from New York. Rehn and Rehn (55) found it parasitizing *Dendrotettix quercus* Pack. in New Jersey. It was reared at Belleville from grasshoppers collected in Prince Edward Island, Quebec, and Ontario.

Life History and Habits

Information on the development and behavior of *T. atlanis* was obtained both from laboratory propagation of the species and from field observations. Host relations were obtained largely from dissections.

Larviposition was not observed in the field. In cages, the female stealthily approaches active or resting grasshoppers and, when sufficiently close, extends the tip of its abdomen forward between its legs and places one or more larvae on the host. These quickly move to a membranous area, perforate it with their mouth parts, and disappear inside. As many as 17 larvae were found in a single field-collected host.

The species is primarily a parasite of adult grasshoppers. Third and fourth instar hosts are rarely parasitized and fifth instar only occasionally.

Howitt (41) observed the adults feeding at flowers of *Symphoricarpos* sp. and *Helianthus annus* L., and on the honeydew excreted by aphids infesting *A. cana*.

Data on the life history of *T. atlanis* as determined from laboratory propagation at 24° C. are as follows:

	Max.	Min.	Mean
Number of larvae per female	97	5	44.6
Larval development in host (days)	21	4	7.4
Days ex host as mature larva	9	1	2.5
Days in puparium	24	8	11.5
Days from deposition of larva to adult	34	17	20.9
Gestation period, approximately (days)			9
Ratio of females to males		53.3 : 46.7	

The mature larva either proceeds with its development to the adult stage after leaving the host or enters diapause. Diapause requirements are not yet known. In some instances pupation and development to the adult took place after an exposure to a temperature of 0° C. for 36 days; in others, as many as 72 days at this same temperature was not effective. Larvae held at 8° C. for as many as 175 days pupated and produced healthy adults.

The first instar larva (63) and the puparium (36) have been described and figured.

Seasonal Occurrence

T. atlanis is present in the field from early June to late September, probably until the first frost. The species has one or possibly two summer generations and an overwintering one.

FIG. 10. Distribution of *Tephromyiella atlanis* (Ald.) in the four western provinces.

Distribution

The species is widespread and occurs from southeastern Manitoba to central British Columbia, and from the southern border of Canada, north to Drumheller, Prince Albert, and Lettonia (Fig. 10).

Hosts

Aeropedellus clavatus, Chorthippus longicornis; *Camnula pellucida*, and other undetermined Oedipodinae; *Melanoplus angustipennis, M. confusus, M. dawsoni, M. femur-rubrum, M. mexicanus, M. packardii*, and *Phoetaliotes nebrascensis.* Dead larvae were found in *Ageneotettix deorum* and *Spharagemon collare* (Scudd.).

Incidence of Parasitism

Though adults of *T. atlanis* were present in the field as early as June, the occurrence of parasitized grasshoppers was rare before late July. Parasitism usually increased to a maximum in early September, when the grasshoppers were largely in the adult stage.

The highest parasitism recorded for *T. atlanis*, 54.5%, was found in a small collection of *M. angustipennis* taken at Asquith, Saskatchewan, August 31, 1949. The approximate proportions of collections with this species were as follows: *M. bivittatus*, 10%; *M. femur-rubrum*, 10%; *C. pellucida*, 10%; *M. packardii*, 20%; and *M. mexicanus*, 25%.

Discussion

T. atlanis is a late-season parasite and occurs in association with *P. hunteri.* It is a relatively persistent parasite of *M. mexicanus* but its incidence rarely exceeds 15.0% of the total parasitism of this species. It is also one of the few parasites that attacks *C. pellucida* and in certain areas it accounts for 50.0% of the total parasitism of that species. In the 6-year period 1948 to 1953, *T. atlanis* accounted for 30.0% of total parasitism in the acridids at Asquith and was relatively more important in that locality than in any other.

Though *T. atlanis* is a familiar inhabitant of late-season grasshoppers it does not appear to play an important role as a natural mortality factor. It occurs frequently in *M. bivittatus* but is itself killed in this host.

Tachinidae

Before dealing with the tachinid parasites it is necessary to state that the specific identifications of the larvae were not fully satisfactory. The identification of larvae in the dissections was based largely on the descriptions of Smith and Finlayson (63). However, there is considerable variation in the shape of the infrabuccal (63) or buccopharyngeal (58) plate, resulting possibly from variations in the pigmentation or sclerotization of the area. There is also considerable variation in the degree of pigmentation of the scalelike spines. Further, according to Dupuis (33) the larva identified as *Paradionaea atra* (Tns.) by Smith and Finlayson (63) would appear to belong, with the other species, in the Acemyini.

In view of these difficulties, specimens were occasionally marked in the records as *Ceracia* type, *Euacemyia* type, *Hemithrixion* type, or just tachinid.

Rearings from living grasshoppers indicate that *Ceracia dentata* (Coq.) is rare in Western Canada. Tachinids reared from grasshoppers collected in British Columbia and Alberta were almost entirely *Euacemyia tibialis* (Coq.). *E. tibialis*, *C. dentata*, and *Hemithrixion oestriforme* B. & B. were reared from Saskatchewan grasshoppers; there, *C. dentata* was scarce and *H. oestriforme* about one and one half times as abundant as *E. tibialis*. In Manitoba, *E. tibialis* and *H. oestriforme* were present, the latter about twice as abundant as the former.

CERACIA DENTATA (Coq.)

This species was described by Coquillett (25) in 1895 as *Acemyia dentata* and was included in his (26) revision of 1897. Specimens were from Massachusetts, Georgia, Florida, Alabama, Mississippi, Missouri, and California. Its association with the genus *Ceracia* was noted by Aldrich (4) in 1933.

The species was recorded as a parasite of *Chortophaga viridifasciata* as early as 1877 (26). In 1924, it was reported as a parasite of *Xanthippus neglectus* (Thos.) and *C. viridifasciata* in the Nicola Valley, British Columbia, by Treherne and Buckell (80), who also mentioned it as occurring in southern British Columbia, at Bozeman, Montana, and at Ottawa, Ontario. Smith (62) recorded it from *M. mexicanus*, and *M. femur-rubrum* in Ontario. Newton (49) recorded it from *M. packardii*, *M. confusus*, *M. bruneri* Scudd., and *C. pellucida* in Montana, and possibly from *Psoloessa delicatula* (Scudd.) in North Dakota. St. Amand and Cloyd (58) described its development in *C. viridifasciata* in Tennessee.

Life History and Habits

The most complete report on the biology of *C. dentata* is by St. Amand and Cloyd (58). The species was not propagated in the laboratory in the course of the present investigation though this was attempted frequently with adults

obtained from field-collected grasshoppers. These attempts resulted only in the deposition of infertile eggs. The flies were usually short-lived in the laboratory, though one female lived for 34 days at 24° C. Much information on the species was obtained from larvae found in dissections and from adults resulting from rearings.

As stated by Townsend (76, p. 73), the incubated egg is deposited on the grasshopper and attached with a glutinous secretion. The egg is small, macrotype, elongate–oval, well flattened, 0.4 mm. in length, 0.2 mm. in width, and approximately 0.06 mm. in depth. The chorion is thick dorsally, and thin ventrally on the side of attachment to the host. The eggs are placed on all stages of hosts.

Each ovary consists of 12 ovarioles. The eggs pass down the paired oviducts to the common oviduct, into the preuterus, past the ducts of the spermathecal and fecundatory glands and into the uterus. There are three spermathecae. The uterus is a thick-walled tube of about two coils. As many as 200 eggs were found in the uterus and ovaries of one female.

Larvae in incubated, but undeposited eggs, lacked the infrabuccal plate (63) which is present in most early-stage larvae found in grasshoppers. At what stage of larval development this plate first becomes apparent is not known. In a few instances, larvae without the plate or with it only slightly developed were found in grasshoppers. This suggests that the larva with the plate may be the first larval stage and that the plate does not become fully formed and pigmented until after the larva has entered the host.

The first instar larva (second instar of Smith and Finlayson (63)) is free-living in the haemocoele of the host. Later larval stages are associated with a cuplike integumental funnel for respiratory contact with the outside air. The funnel fits snugly around the caudal end of the larva, and its small end is attached to a large trachea close to a spiracle in the thoracic region of the host.

If the free-living larva with the buccal plate is the first instar, there are three larval stages of the parasite in the host rather than four as reported by St. Amand and Cloyd (58).

The full-grown larva pupates shortly after leaving the host. The adult emerges 8 to 13 days (mean 10.6) later at 24° C. or the parasite goes into diapause in the pupal stage.

The early-stage larva was described by Smith and Finlayson (63). All larval stages, and the puparium were described by St. Amand and Cloyd (58), and the puparium was described and figured by Greene (35). Boyes and Wilkes (10) have described and figured the somatic chromosomes.

Seasonal Occurrence

C. dentata is present in the field from mid-June to mid-September, perhaps until the first frost. The species has three summer generations and an overwintering one. It overwinters in the pupal stage.

Distribution

Records on the occurrence of *C. dentata* in Western Canada are not extensive. Specimens in the collections of the Entomology Laboratory, Saskatoon, are

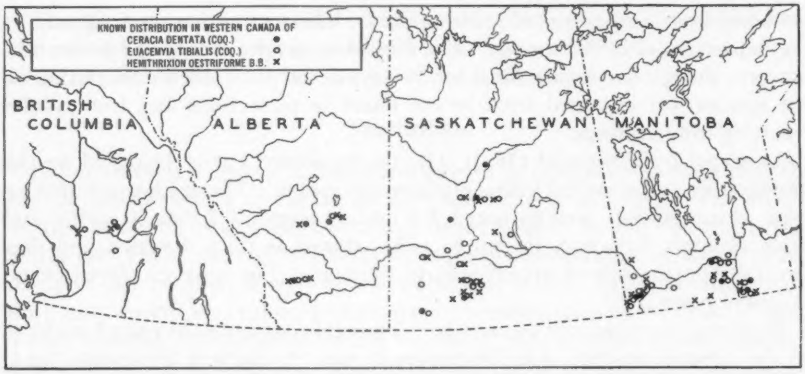


FIG. 11. Distribution of *Ceracia dentata* (Coq.), *Euacemyia tibialis* (Coq.), and *Hemithrixion oestriforme* B. & B. in the four western provinces.

from Saskatoon and Duck Lake. Specimens were reared from collections of grasshoppers from Dollard and Marriott in Saskatchewan, and from Rainbow and Carbon in Alberta. Distribution as determined from rearings, dissections, and field captures is shown in Fig. 11.

Hosts

Aulocara elliotti; *Camnula pellucida*, *Chortophaga viridifasciata* (80), *Xanthippus neglectus* (80); *Melanoplus bivittatus*, *M. femur-rubrum*, *M. infantilis*, *M. mexicanus*, and *M. packardii*.

Incidence of Parasitism

C. dentata was the least important of the three tachinid species found. Of the tachinid adults obtained from collections of living grasshoppers only 1.7% were of this species.

On present evidence, *C. dentata* is not important in the natural control of grasshoppers in Western Canada.

EUACEMYIA TIBIALIS (Coq.)

This species was described by Coquillett (26) in 1897 as *Acemyia tibialis* from a single female collected in the Santa Cruz Mountains, California. In 1912, Townsend (69) proposed the new genus *Euacemyia* with *A. tibialis* as type.

Though the species was relatively abundant as a parasite of grasshoppers, the first published references to it in this role appear to be those of Smith (59) in 1940 and Smith (62) in 1944, who reported it as a parasite of grasshoppers in Manitoba, Ontario, and Quebec. Newton (49) reared it as a parasite of *M. bruneri* in Park County, Montana, in 1953, and stated that it was apparently of minor importance as a grasshopper parasite.

Life History and Habits

Larvae of *E. tibialis* were reared frequently from collections of grasshoppers, but laboratory propagation of the species was not successful.

The reproductive system is similar to that of *C. dentata*, described above, and the manner of parasitizing and developing in the host are no doubt the same. As many as 150 eggs were found in a female. All host stages are parasitized. The full-grown larva pupates shortly after leaving the host. The adult emerges 9 to 17 days (mean 13.2) later at 24° C. or the parasite remains in diapause in the pupal stage. Once in diapause, the minimum time from pupation to emergence of the adult was 135 days. Cold treatments were not effective in shortening the developmental period below this minimum.

The early-stage larva with the infrabuccal plate was described previously by Smith and Finlayson (63). The puparium is apparently indistinguishable from that of *Coquillettina plankii* Walt. as described by Greene (35).

Seasonal Occurrence

E. tibialis is present in the field from mid-June to late September. It may have three summer generations and an overwintering one but as approximately 45.0% of the pupae from the collections entered diapause the number of generations in the field is uncertain. It overwinters in its puparium in the soil.

Distribution

The species is widespread and occurs from southeastern Manitoba to British Columbia, and north from the Canadian border to Drumheller, Saskatoon, and Rosser (Fig. 11).

Hosts

Aeropedellus clavatus, *Aulocara elliotti*, *Chorthippus longicornis*; *Camnula pellucida*; *Melanoplus angustipennis*, *M. bivittatus*, *M. borealis junius*, *M. confusus*, *M. dawsoni*, *M. femur-rubrum*, *M. infantilis*, *M. keeleri luridus* (Dodge), *M. mexicanus*, *M. packardii*, and *Phoetaliotes nebrascenis*.

Incidence of Parasitism

Parasitism was extensive, and judged from dissections this was the predominant tachinid parasite of grasshoppers in Western Canada. However, as mentioned previously, the separation of *E. tibialis* from *H. oestriforme* in the larval stages was unsatisfactory, and the evidence from rearings is that these two species were about equally abundant or that *H. oestriforme* predominated.

Highest parasitism by *E. tibialis*, 56.3%, based on dissections, was found in *M. packardii* collected at Glenbain, Saskatchewan, September 13, 1950. The approximate proportions of collections with this species were as follows: *C. pellucida*, 16%; *M. mexicanus*, 32%; *M. packardii*, 48%; *M. femur-rubrum*, 55%; and *M. bivitattus*, 65%.

Discussion

E. tibialis is a common parasite of grasshoppers and an important natural control agent. *M. bivittatus* and *M. mexicanus* are preferred hosts, followed by *M. femur-rubrum* and *M. packardii* in the areas where they occur. Few grasshopper species were free from its attack.

E. tibialis undergoes little mortality in any grasshopper species, including *M. bivittatus*, a host in which many other parasites succumb. The host may remain active for a time following emergence of the fully grown tachinid larva, but the reproductive organs are either destroyed or, in the females, sufficiently damaged to prevent or greatly reduce oviposition.

HEMITHRIXION OESTRIFORME B. & B.

This species was described by Brauer and Bergenstamm (12) in 1891; the holotype male was from Colorado. The species was not recognized by Coquillett (26) in his 1897 revision, but was included by him (27) in 1910 with his type species of the North American genera of Diptera. Curran (28) recognized the genus in 1934. In 1939 Townsend (79, p. 262) stated that the species was recorded only from the Rocky Mountain region. Smith (62) reported it in 1944 from *M. bivittatus* and *M. mexicanus* in Manitoba, and Newton (49) found it as a parasite of *M. bivittatus*, *M. bruneri*, *C. pellucida*, and *Boopedon nubilum* (Say) in Montana in 1953.

Life History and Habits

Little is known of the life history of *H. oestriforme*. It was not propagated successfully in the laboratory, though many adults were reared from grasshoppers.

Its reproductive system is similar to that of *C. dentata* and its habits and development are probably similar to those of the two species of tachinids discussed above. The type and number of larval stages in the host are the same as in those species. It parasitizes all host stages.

Fully grown larvae emerged from grasshoppers 3 to 19 days after the hosts were collected, and the duration of the pupal stage was 9 to 20 days (mean 11.3). Diapause occurs in the pupal stage, but unlike *E. tibialis*, less than 10.0% of the puparia obtained from grasshoppers entered diapause. Pupae in diapause for 131 days at 24° C. produced adults at this same temperature 13 days after an exposure to 8° C. for 17 days.

Seasonal Occurrence

Adults are present in the field from mid-June to late September. There are two or three summer generations and an overwintering one. The winter is passed in the puparium in the soil.

Distribution

The species is widespread throughout southern Manitoba and Saskatchewan and as far north as Saskatoon. It was not reared from grasshoppers collected in Alberta or British Columbia, but larvae, identified as of this species, were found in dissections of grasshoppers from these two provinces (Fig. 11).

Hosts

Chorthippus longicornis; *Camnula pellucida*; *Melanoplus bivittatus*, *M. femur-rubrum*, *M. infantilis*, *M. mexicanus*, and *M. packardii*.

Incidence of Parasitism

Parasitism by *H. oestriforme* rarely exceeded 5.0%. The maximum recorded for the species, 18.2%, was found by dissection in a collection of *M. bivittatus* made at Fortier, Manitoba, in 1948. However, it is evident from rearings that *H. oestriforme* was equal to *E. tibialis* in abundance, and therefore more important than the dissection records indicate. It was the most abundant tachinid parasite of grasshoppers in the Gravelbourg, Hodgeville, and Esme areas of southern Saskatchewan, and an important natural control agent.

Muscidae

ACRIDOMYIA CANADENSIS Snyder

This small muscid was described by Snyder (64) in 1940; the holotype male and allotype female were reared from *M. bivittatus* collected at Arnaud, Manitoba, in 1938 in the course of the present investigations. One female was collected by King at Saskatoon in 1924 (64). Newton (49) reported the species as a parasite of grasshoppers in Montana in 1952 and 1953.

Life History and Habits

Oviposition by this species was observed in the laboratory in 1939, but with a few exceptions propagation of the species was never successful.

The life history and behavior of *A. canadensis* are no doubt similar in many respects to those of *A. sacharovi* Stack., described by Rukavishnikov (57) in 1930 as a parasite of the migratory locust in Kazakstan.

The female punctures the host integument with its rasping mouth parts and feeds upon the body fluids. The puncture serves also as a point of insertion for the ovipositor. The tip of the ovipositor is barbed and, when inserted, the female is not easily dislodged by movements of the host. The host is little disturbed by the puncturing, feeding, and ovipositing of the parasite.

The egg, 0.5 mm. in length, 0.2 mm. in diameter, and cream to white in color, is placed directly into the haemocoele of the host. Occasionally, clusters of eggs lodged among the muscle fibers are visible through the integument of the host. The average number of eggs produced by *A. sacharovi* is 200 (57) and the number produced by *A. canadensis* is perhaps similar.

There are three larval stages, each free-living in the haemocoele of the host. The first instar larva is readily distinguished by the shape of the mouth-parts (63) and the second and third instars by the form of the anterior and posterior spiracles, which are widely spaced, almost circular in form, and with radiating papillae.

The larvae of *A. canadensis* are gregarious and as many as 70 were found in a single host. Larvae were found in third instar to adult hosts.

The fully grown larva pupates shortly after leaving the host. All puparia obtained from rearings entered diapause and none yielded adults in the same year. In this respect *A. canadensis* differs from *A. sacharovi*, which has two summer generations (57).

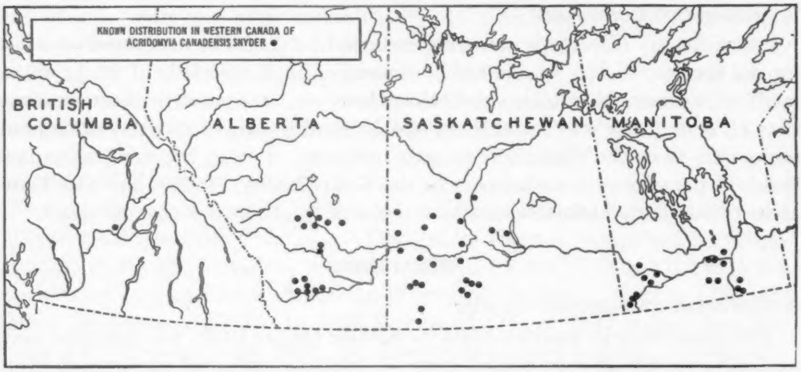


FIG. 12. Distribution of *Acridomyia canadensis* Snyder in the four western provinces.

Various cold treatments were not effective in hastening emergence of *A. canadensis* from their puparia. Adults were obtained from puparia as many as 637 days old.

Rukavishnikov stated that the females of *A. sacharovi* mature rapidly and are ready to lay eggs 3 to 5 days after eclosion. Larvae of *A. sacharovi* mature in 11 to 14 days, and the duration of the pupal stage is 22 to 26 days.

Seasonal Occurrence

A. canadensis is present in the field from mid-June to late September. It has one generation a year and overwinters in the pupal stage.

Distribution

A. canadensis occurs from southeastern Manitoba to Kamloops, British Columbia, and north from the Canadian border to Drumheller, Saskatoon, and Winnipeg (Fig. 12).

Hosts

***Ageneotettix deorum*, *Aulocara elliotti*; *Camnula pellucida*, *Spharagemon* sp.; *Melanoplus angustipennis*, *M. bivittatus*, *M. dawsoni*, *M. femur-rubrum*, *M. infantilis*, *M. mexicanus*, *M. packardii*,** and ***Phoetaliotes nebrascensis*.**

Incidence of Parasitism

Maximum parasitism recorded for the species, 68.8%, occurred in *M. packardii* collected at Glenbain, Saskatchewan, in 1950. The species was more common and relatively more important in Saskatchewan and Alberta than in Manitoba. In Manitoba, approximately 10.0% of the total parasitism of *M. bivittatus* was caused by *A. canadensis*; in Saskatchewan, approximately 35.0%; and in Alberta, about 30.0%.

The approximate proportions of collections with this parasite were as follows: *C. pellucida*, 10%; *M. mexicanus*, 17%; *M. bivittatus*, 30%; and *M. packardii*, 36%.

Discussion

A. canadensis is a midseason to late-season species and is primarily a parasite of fifth instar and adult grasshoppers. It shows some preference for *M. bivittatus* and *M. packardii* when these species occur, but also parasitizes other species rather heavily.

In spite of the smallness of the larvae of this parasite the evidence from the dissections is that few hosts survive the presence of more than two or three larvae.

A. canadensis is of most value as a natural control factor in Saskatchewan and Alberta.

Nemestrinidae

Two species of Nemestrinidae are known to attack grasshoppers in Western Canada: *Trichopsidea* (*Parasymmictus*) *clausa* (O.S.), and *Neorhynchocephalus sackenii* (Will.). Spencer (67) stated that both occur in the Lac du Bois area of British Columbia, where *T. clausa* was reared chiefly from *C. pellucida*, and *N. sackenii* from *M. mexicanus*. York and Prescott (81) reported the same two species as parasites of grasshoppers in Montana. *T. clausa* was reared by them from *Metator pardalinus* (Sauss.), and from *Arphia pseudonietana* (Thos.); *N. sackenii* was reared from *Encoptolophus sordidus costalis* (Scudd.), *M. mexicanus*, *M. alpinus* Scudd., *M. dawsoni*, and *M. infantilis*. Several other grasshopper species were found infested with nemestrinid larvae. Up to 80.0% of certain hosts were reported as infested. Bequaert (8) reported the occurrence of *N. sackenii* in Iowa and Missouri, and Cole and Lovett (23) reported it in Oregon.

Papers by Bequaert (5, 6, 7) may be consulted for a systematic treatment of the group.

Life History and Habits

The nemestrinids were not observed in the field during the present investigation. A few adults were reared from field-collected larvae, and several hundred first instar larvae were obtained from field-collected eggs. Larvae in various stages were found in dissections of grasshoppers.

Observations on the behavior of the flies in the field were reported by Spencer (65), York and Prescott (81), and Dietz (31), and more recently by Prescott (54). Observations on the development of the immature stage were reported by Prescott (54), and York and Prescott (81), and the morphological features of the immature stages of *Neorhynchocephalus* species in Argentina were described and figured by de Crouzel and Salavin (30).

Briefly, the life history of these flies, as described in the literature, is as follows: The species overwinters in the soil as a mature larva. The larva pupates in the spring and the adult emerges in late May to mid-July. After mating, the female lays its eggs in any available crevices or holes in weeds or timber, or under the loose bark of living trees. The flies of *N. sackenii* appear to be more selective than those of *T. clausa* and choose only beetle holes for

their eggs (54). One female of *T. clausa* was observed (81) to deposit as many as 4700 eggs in a 7-hour period. The eggs are about 0.5 mm. in length, and light cream in color. They hatch in 8 to 10 days and produce larvae that are similar in size and color to the eggs. These larvae survive 5 to 14 days and are probably scattered by the wind after they crawl from the cracks. What happens from this time until they appear in the grasshoppers, and how they contact the host is not known. Prescott (54) has reported in considerable detail differences in the habits of the larvae of these two nemestrinids. He observed that the larvae of *N. sackenii* enter the host through the soft intersegmental folds of the abdominal venter slightly below and posterior to a spiracle whereas the larvae of *T. clausa* gain entry to their hosts by puncturing the tracheae just inside the thoracic or first abdominal spiracles.

According to Spencer (67), the nemestrinids have four larval instars and all except the first instar breathe by means of a tracheal sheath or funnel attached to a thoracic trachea of the host. According to Prescott (54) the funnel of *N. sackenii* is attached to the abdominal wall of the host and the manner of attachment serves to distinguish the species. This funnel or tube is a characteristic feature of the nemestrinids and often exceeds the larva in length. The caudal end of the larva, with its posterior spiracles, fits into the enlarged end of the funnel. The larval exuviae remain attached to the funnel, that of the first instar larva is found within the first 1.0 mm. stretch of the funnel.

No information is available on the time required for development of the larvae in the host. According to York and Prescott (81), mature larvae proceeded with their development after 2 months' exposure to below-freezing temperatures. De Crouzel and Salavin (30) reported that species of *Neorhynchocephalus* remained in diapause from 43 to 1011 days. The pupal stage, according to these observers, lasts from 10 to 32 days. The pupa is active and works its way to the surface of the soil prior to eclosion of the adult.

The adults were short-lived in the laboratory and rarely survived more than 2 or 3 days.

Nemestrinid larvae were found in fifth instar and adult grasshoppers only. Prescott (54) found larvae of *N. sackenii* in fourth instar nymphs of *M. mexicanus*.

Seasonal Occurrence

Nemestrinid larvae were present in grasshoppers from early July to mid-September. Spencer (66) reported adults at Riske Creeke, British Columbia, as early as June 3, and this apparently is the most northerly point known for representatives of this family in the world (67). York and Prescott (81) observed adults in Montana from June 13 to late August. They are probably on the wing in Canada from early June until late August.

Distribution

The nemestrinids were not found east of Lyleton in southwestern Manitoba. They occurred west from there to British Columbia, but were of infrequent occurrence in grasshoppers except in the latter province (Fig. 13).

Hosts

Nemestrinids were dissected from the following hosts in Western Canada. *Aeropedellus clavatus* and unidentified Acridinae; *Camnula pellucida*, *Sphara-gemon* sp., and unidentified Oedipodinae; *Melanoplus angustipennis*, *M. confusus*, *M. infantilis*, and *M. mexicanus*. Records of occurrence in *Sphara-gemon* sp. and in *M. infantilis* are based on the presence of dead larvae in these hosts. From host records in Montana (81, 82) and Oregon (53) it appears likely that *Ageneotettix deorum*, *M. bivittatus*, and *M. femur-rubrum* could be added to the list of Canadian hosts.

During the present investigation no distinction was made between the larvae of *T. clausa* and *N. sackenii*.

Incidence of Parasitism

Maximum parasitism recorded for the nemestrinids in the present investigation, 20.9%, occurred in *C. pellucida* collected at Lillooet, British Columbia, in 1952. Only a small percentage of the collections made in the course of the investigations contained these parasites.

In British Columbia, where the nemestrinids were most conspicuous, they accounted for 12.0 to 70.0% of the total parasitism.

The present investigations indicate that the nemestrinids are not important parasites in the prairie regions of Canada. The high percentages of parasitism produced by them in Montana (81, 82) and Oregon (53) suggest that they might at times be important parasites in Canada of the Acridinae and Oedipodinae, and of *M. mexicanus* and *M. bivittatus*.

Mermithidae

According to Christie (19) the mermithid parasites found most commonly in grasshoppers are *Agamermis decaudata* Cobb, Steiner, & Christie, and *Mermis subnigrescens* Cobb.

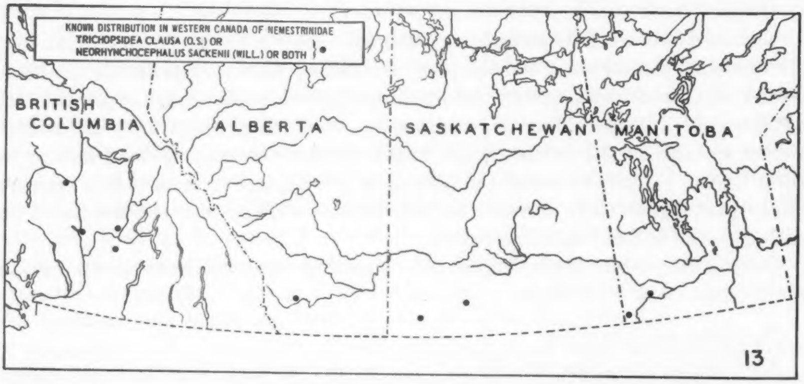
These two species are presumably the ones present in Western Canada, though no attempt was made to distinguish between them. They were found in the immature stages, in which they are difficult, if not impossible, to identify to species.

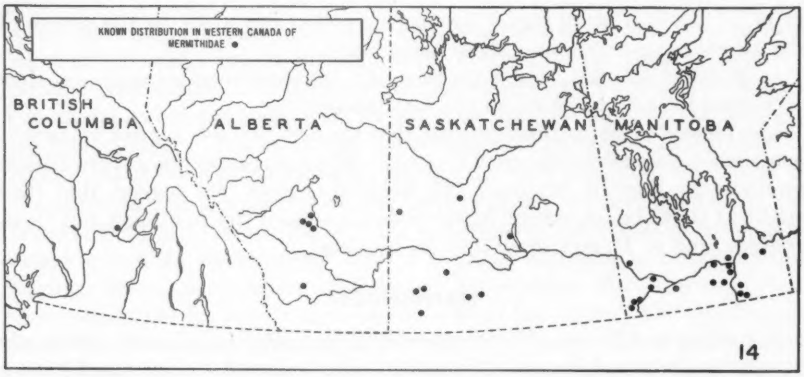
Glaser and Wilcox (34) reported a *Mermis* epidemic among grasshoppers in southern Vermont in 1917 where as many as 76.0% of *M. bivittatus* were infested with nemas in early September. Hayes and DeCoursey (39) reported a 14.0% infestation of grasshoppers, mostly *M. differentialis* and *M. femur-rubrum*, by nematodes near Urbana, Illinois, in 1937.

Life History and Habits

Information on the life history and morphology of these two species is given by Cobb (21), Cobb, Steiner, and Christie (22), and Christie (18, 19, 20), whose papers may be consulted for further details.

Briefly, both species have a 2 year life cycle but differ considerably in other respects. The eggs of *A. decaudata* are deposited in the soil, and under suitable conditions of moisture the young nemas that hatch from them migrate to the





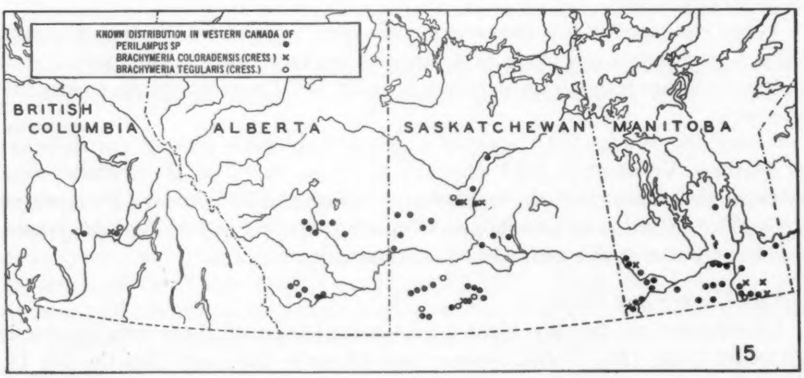


FIG. 13. Distribution of Nemestrinidae in the four western provinces.
FIG. 14. Distribution of Mermithidae in the four western provinces.
FIG. 15. Distribution of the secondary parasites *Perilampus* sp., *Brachymeria coloradensis* (Cress.), and *Brachymeria tegularis* (Cress.) in the four western provinces.

vegetation, await the arrival of a host, attach themselves to it, and penetrate to the haemocoele by means of their sharp oral tooth or stylet. The eggs of *M. subnigrescens* are deposited on the vegetation and are ingested by feeding grasshoppers. They hatch in the alimentary tract of the host and the nemas gain entry to the haemocoele through the walls of the digestive tract.

Both species require 1 to 3 months to complete their development in the grasshopper. Each leaves the host late in the season and overwinters in the soil at a depth of 2 to 10 in. Each attains maturity while in the soil and produces infective larvae or eggs the second summer after it enters the soil. A female of each species produces more than 10,000 eggs. The eggs of *M. subnigrescens* are very durable and remain viable throughout the summer.

A. decaudata is considered a parasite of grasshoppers and other insects, and *M. subnigrescens* a parasite of grasshoppers only.

Nemas were found in second nymphal to adult hosts.

Seasonal Occurrence

Nemas were found in grasshoppers from early June to late September. Infective larvae and eggs are presumably present in the field from May until the first frost (19, 20).

Distribution

The mermithidae are widely distributed in Western Canada from eastern Manitoba to Kamloops in British Columbia. They occur from the Canadian border north to Morrin, Saskatoon, and Oak Hammock (Fig. 14).

Hosts

Aeropedellus clavatus, *Chorthippus longicornis*, and unidentified Acridinae; *Camnula pellucida* and unidentified Oedipodinae; *Melanoplus bivittatus*, *M. dawsoni*, *M. femur-rubrum*, *M. gladstoni*, *M. infantilis*, *M. mexicanus*, *M. packardii*; *Conocephalus saltans* (Scudd.) and unidentified Tettigoniidae; and *Acheta assimilis* F.

Incidence of Parasitism

Maximum parasitism by nemas, 36.0%, was found in *M. mexicanus* collected at Rosser, Manitoba, on July 15, 1943. In general, parasitism by mermithids was less than 10.0%, and except at Arnaud, Manitoba, and Carbon, Alberta, where they were frequently present, the occurrence of these nematodes was irregular and uncertain.

Discussion

The mermithids do not appear to be of great importance in natural control in the localities surveyed in Western Canada. However, with the exception of two localities in Alberta, Granum and Stavely, mermithids were present occasionally in collections.

Christie (20) believed that *M. subnigrescens* was the more important parasite of the two, that it was able to withstand a greater variety of soil and climatic conditions than *A. decaudata*, and able to maintain itself in large numbers where grasshopper populations are consistently low. He also stated that

semi-arid conditions may prevent the spread of the mermithids. It is probable that the sporadic occurrence of the mermithids in many parts of Western Canada is due to the dryness of the areas.

Perilampidae and Chalcididae

No list of the parasites of nymphal and adult grasshoppers in Canada would be complete without mention of the secondary parasites. Adults of the species concerned were identified by the systematists of the Insect Systematic and Biological Control Unit at Ottawa as *Perilampus* sp. (*hyalinus* Say group), *Brachymeria coloradensis* (Cress.), and *B. tegularis* (Cress.). These secondary parasites are at times relatively abundant and must be an important cause of mortality among the primary parasites.

PERILAMPUS sp. (HYALINUS Say group)

The life history and habits of this species (or group) were not studied extensively. They were described in some detail by Smith (61) in 1912. At that time Smith was unable to explain how the first stage larvae (planidia) came to be located on the skin of the caterpillars upon which they were found. This question has now been answered with respect to grasshoppers.

Females in cages in the laboratory laid eggs on blades of grass. Up to 600 eggs were laid by one caged female, and it is probable that females in the field are more fecund. The planidia that hatch from these eggs lie in wait for the arrival of a grasshopper, occasionally standing erect on their caudal ends and waving themselves about. Once attached to a host they penetrate to the haemocoele through a membranous area. In the laboratory, planidia were observed to penetrate the pulvilli of grasshoppers.

Though only 0.3 mm. in length, the planidium is easily seen in careful dissections of grasshoppers by reason of its dark color, and is also easily visible through the semitransparent integument of dipterous larvae. It is not injurious to the grasshopper, and is dependent on the presence of a larva of a primary parasite for further development.

If the larva of a primary parasite is not already present, the planidium remains in wait, undeveloped, in the haemocoele of the grasshopper. When a parasite is present, the planidium enters it but remains in the planidial stage until the host has formed its puparium. During the course of host histolysis the planidium, according to Smith (61), orients itself in such a way as to be external to the dipterous pupa when pupation is completed, and becomes ectoparasitic within the puparium. It completes its development as an ectoparasite and emerges from the host puparium as an adult.

Emergence of *Perilampus* was usually 20 to 30 days later than that of a similar but unparasitized host.

Planidia of *Perilampus* were found in all stages of grasshoppers except the first nymphal instar. As many as 23 planidia were found in one grasshopper. Occasionally several were found in dipterous larvae, but only one *Perilampus* adult emerges from a puparium.

Perilampus overwinters in both host larvae and puparia.

Seasonal Occurrence

Planidia were present in grasshoppers from late June until frost. The number of generations per season depends entirely on the length of time the parasite takes to contact a grasshopper and upon the chance arrival of a dipterous host. It also depends on whether the dipterous host enters diapause or proceeds with its development without delay.

Distribution

Perilampus sp. is widespread and occurred, with few exceptions wherever grasshoppers were collected (Fig. 15).

Hosts

Planidia of *Perilampus* were found in larvae of the following grasshopper parasites: *Blaesoxiphotheca coloradensis, Protodexia hunteri, Sarcophaga* sp. C, *Sarcophaga* sp. H, *S. reversa, Tephromyiella atlanis, Euacemyia tibialis*, and *Acridomyia canadensis.*

Perilampus planidia were found in the following grasshoppers in Western Canada: *Aeropedellus clavatus, Ageneotettix deorum, Aulocara elliotti, Chorthippus longicornis*; *Camnula pellucida, Encoptolophus sordidus costalis*; *Aeolopus turnbulli bruneri* Caudell, *Hesperotettix viridis pratensis* Scudd., *Melanoplus angustipennis, M. bivittatus, M. dawsoni, M. femur-rubrum, M. gladstoni, M. infantilis, M. keeleri luridus, M. mexicanus, M. packardii, Phoetaliotes nebrascensis*; *Conocephalus saltans, Orchelimum gladiator* Bruner, *Scudderia* sp.; and *Oecanthus nigricornis* Wlk.

Incidence of Infestation

Highest infestation by *Perilampus* sp., 85.7%, was found in *M. bivittatus* collected at Stavely, Alberta, September 15, 1947; 65.8% of the specimens of *M. mexicanus* collected at Elva, Manitoba, September 2, 1946, also contained this secondary parasite.

Relatively high infestations of *Perilampus* recorded in grasshoppers collected in additional localities, or in other hosts in Manitoba, were as follows:

Locality	Host	Period collected	% infestation
Greenridge	*M. dawsoni*	Aug. 16–31, 1941	55.0
	M. packardii	Aug. 16–31, 1941	47.3
Graysville	*M. bivittatus*	Aug. 1–15, 1951	46.1
	M. mexicanus	Aug. 16–31, 1941	40.0
	M. packardii	Aug. 16–31, 1941	47.2
Glenboro	*M. femur-rubrum*	Aug. 1–15, 1941	41.2
	M. mexicanus	Aug. 1–15, 1951	58.5
	C. saltans	Aug. 1–15, 1951	66.7
Brandon	*M. bivittatus*	Sept. 1–15, 1942	42.3
Souris	*M. packardii*	Aug. 16–31, 1941	52.4
Elva	Tettigoniidae	July 16–31, 1946	60.9
	Tettigoniidae	Aug. 1–15, 1947	40.0

The approximate proportions of collections with this species were as follows: *C. pellucida*, 10%; *M. femur-rubrum*, 30%; *M. bivittatus* and *M. mexicanus*, 35%; and *M. packardii*, 40%.

Discussion

It is quite apparent that in seasons when the incidence of *Perilampus* is high the survival and reproduction of the primary parasites may be seriously affected, for it is certain that few, if any, dipterous larvae in grasshoppers with *Perilampus* ever reach the adult stage.

Perilampus in field-collected grasshoppers used for the laboratory propagation of parasites on one occasion caused an 80.0% mortality of the resulting dipterous larvae. Even more surprising was the establishment of *Perilampus* planidia in larvae of *Kellymyia kellyi* while the latter were feeding on the bodies of freshly-crushed grasshoppers. *K. kellyi* larvae complete their feeding within 3 days and the planidia had little time to find and enter these active larvae before the grasshoppers had dried and were abandoned.

BRACHYMERIA COLORADENSIS (Cress.)

Information on this secondary parasite is limited largely to records of its occurrence in parasites reared from grasshoppers. Its life history was not investigated but is presumed to be similar to that of *Brachymeria compsilurae* (Cwfd.), described by Dowden (32).

Kelly (43) reported it as a parasite of *K. kellyi* in Kansas in 1914. Peck (52) gave its distribution as Ontario, North Carolina to Florida, Texas, and Saskatchewan. Burks (16) considered *B. coloradensis* a secondary parasite of grasshoppers and noted that it had been reared in America, Eastern Siberia, Russia, and Turkestan.

Adults of *B. coloradensis* were reared from both sarcophagid and tachinid puparia, but no larvae of this species were found in grasshoppers in the course of dissections. The closely related *B. compsilurae* (32) parasitizes dipterous larvae while they are within their lepidopterous hosts, and a similar habit by *B. coloradensis* would explain why the larvae of the latter were not found in grasshoppers. If subsequent development of the two species is similar, *B. coloradensis* remains in its first larval stage until the dipterous host has formed its puparium. It then proceeds with its development inside the puparium, presumably as an ectoparasite, and later emerges as an adult. If the host is not in diapause, *B. coloradensis* emerges 17 to 19 days after the host puparium has been formed, and about five to seven days later than the adult of a similar but unparasitized host.

B. coloradensis overwinters in the mature host larva or puparium, presumably as a first stage larva in a host larva, and as a full-grown larva in a host puparium.

It occurs in the field from early July to mid-September. It is widespread and occurs from southeastern Manitoba to Kamloops, British Columbia (Fig. 15).

Specific identification of host puparia was not attempted. However there is no evidence that the parasite discriminated between sarcophagid and tachinid hosts.

Highest parasitism, 35.7%, was found in 14 puparia from a collection of *M. mexicanus* taken at Lyleton, Manitoba, August 19, 1938. As a secondary parasite of grasshoppers it was the predominant *Brachymeria* species in Manitoba.

BRACHYMERIA TEGULARIS (Cress.)

Information obtained on *B. tegularis* is similar to that for *B. coloradensis*, and except for taxonomic differences in the adult stage was not distinguishable from that species.

B. tegularis occurs in the field from mid-June to mid-September. It is widespread, and occurs from eastern Manitoba to Kamloops, British Columbia.

Burks (17) judged from the locality records in Illinois that *B. tegularis* was associated with ordinary grassland and field-crop grasshoppers, whereas *B. coloradensis* was present only in very dry areas where the sand-loving grasshoppers occur. In the present study both species of *Brachymeria* were occasionally obtained from the same collection and often from the same locality and no marked difference in the distribution of the species was apparent. However, *B. tegularis* was more common in Saskatchewan and Alberta than *B. coloradensis*, and it was more frequently a parasite of tachinids than of sarcophagids.

Highest parasitism by *B. tegularis*, 31.8%, was found in 22 puparia from a collection of grasshoppers taken from the Lac du Bois ranges, British Columbia, on July 15, 1953.

For distribution of *B. tegularis* see Fig. 15.

Acknowledgments

The indispensable co-operation of officers of the laboratories of the Entomology Division in Western Canada in collecting grasshoppers and providing sorted material for the survey of parasitism is gratefully acknowledged. Dr. A. J. Howitt, now of the Agricultural Experiment Station, Puyallup, Washington, also participated in the collecting and sorting of western material and carried out various field observations on the parasites in Saskatchewan.

The contribution of Mrs. L. R. Finlayson to the work carried out at Belleville, including her share in the critical examination of grasshoppers, the recognition of new species, and the routine rearing of grasshoppers and propagation of parasites is also gratefully acknowledged. Many of these duties were more recently carried out by Miss Lois Rollins with the assistance of Mrs. H. E. Welch and Miss Frances Gorham.

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*Includes key to genera of sarcophagids, but not referred to in text.

NOTES

RELAXATION AND FIXATION OF ACANTHOCEPHALA[1]

PAUL L. MONTREUIL

A technique described by Bailenger and Neuzil (1) for relaxation of helminths is admirably suited for use with Acanthocephala. It has been used with slight modifications on all routine collections made at this laboratory during the last 18 months, and has a number of advantages over the usual methods.

Bailenger and Neuzil's relaxing solution is made up as follows: menthol 0.25 g., Tween 80* 5 g., water to make 100 cc.

Its action depends on the anaesthetic properties of menthol which is six times more concentrated here than in the saturated aqueous solution often used for relaxing delicate invertebrates. Acanthocephala placed in this are rapidly anaesthetized and soon become turgid, with the proboscis and posterior extremity extroverted. A treatment of less than 30 minutes usually suffices for several species of *Corynosoma*, *Polymorphus*, *Bolbosoma*, *Centrorhynchus*, *Echinorhynchus*, and other genera encountered here. Some of the larger species occasionally require slightly longer treatment and refrigeration, to bring them to a satisfactory state of extrusion.

The same authors recommend the use of Demke's (2) fixative, which is compounded of: formalin 5 cc., acetic acid 5 cc., glycerin 10 cc., ethyl alcohol 24 cc., distilled water 46 cc.

Bailenger and Neuzil suggest flooding the parasite with boiling Demke's solution: I have found this treatment too drastic with *Corynosoma*, *Polymorphys*, and *Echinorhynchus* spp., as it often causes severe "blistering". The fixative is consequently used cold. Specimens should remain in the fixing solution at least two hours; we find it satisfactory to leave material in the fixative for long periods instead of transferring to alcohol or other preservatives.

The advantages of this relaxation and fixation technique are such that at this laboratory all routine collections of Acanthocephala are now treated as described. Relaxation and extrusion of the proboscis is effected much more rapidly than it is following the usual cold-water treatment. Strains and other reagents seem to penetrate the worm more rapidly, possibly owing to the action of the surfactant (Tween 80) in the anaesthetic solution. The fixative is easy to use and does not require extensive washing-out, and material may be stored in it for long periods. However, the greatest advantage of the fixative

[1]No. 65, Contributions du Départment des Pêcheries.
*Polyoxyethylene (20) Sorbitan Monooleate marketed by Atlas Powder Co. of Canada, Ltd., Brantford, Ont.

is that it has a partial clearing action which is sufficient to permit examination of internal structures without separate clearing. This is an important consideration where numerous specimens are to be examined.

It is of interest to note that in fresh collections of *Corynosoma* spp. from seals, treated by the above method, the lemnisci of the males assume a slight pinkish tinge. I have never observed this in females of the same collections, nor in either sex treated by other methods. The color can be observed shortly after fixation, but it disappears after a few days' storage in the fixative.

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Grindstone, Magdalen Islands, Que.

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